THE STUDY OF PREGNANT WOMEN WITH DIABETES AND SCREENING FOR MUTATIONS IN A PANEL OF TEN MODY (MATURITY ONSET DIABETES OF THE YOUNG) GENES AND POLYMORPHISMS IN TCF7L2.

Dissertation submitted to
The Tamil Nadu Dr. M.G.R Medical University,

in partial fulfillment of the rules and regulations for the degree of DM in Endocrinology, August-2014

Christian Medical College, Vellore.

THE STUDY OF PREGNANT WOMEN WITH DIABETES AND SCREENING FOR MUTATIONS IN A PANEL OF TEN MODY (MATURITY ONSET DIABETES OF THE YOUNG) GENES AND POLYMORPHISMS IN TCF7L2.

SECTION I:
NEXT GENERATION SEQUENCING FOR SCREENING FOR MUTATIONS IN TEN MODY GENES IN PREGNANT WOMEN WITH DIABETES.

SECTION II:
THE STUDY OF COMMON TCF7L2 POLYMORPHISMS IN GESTATIONAL DIABETES

BY

DR. D.M.MAHESH

Dissertation submitted to the

THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY,

In partial fulfillment of the requirements for the degree of
DM in ENDOCRINOLOGY

Under the Guidance of

Prof. Nihal Thomas

DEPARTMENT OF ENDOCRINOLOGY,DIABETES AND METABOLISM, CHRISTIAN MEDICAL COLLEGE, VELLORE, TAMIL NADU.

DECLARATION

I hereby declare that this dissertation titled **"The study of pregnant women with diabetes and screening for mutations in a panel of ten MODY (Maturity Onset Diabetes of the Young) genes and polymorphisms in TCF7L2"** was carried out by me under the direct supervision and guidance of Dr. Nihal Thomas, Professor and Head, Department of Endocrinology, Diabetes & Metabolism at Christian Medical College, Vellore.

This dissertation is submitted to The Tamil Nadu Dr.M.G.R University, in partial fulfilment of the requirements for the degree of DM in Endocrinology.

I also declare that this dissertation has not been submitted by me to any other university, or for the award of any other degree.

Vellore Dr.D.M.Mahesh

Date: Christian Medical College, Vellore

CERTIFICATE

This is to certify that this research work titled **"The study of pregnant women with diabetes and screening for mutations in a panel of ten MODY (Maturity Onset Diabetes of the Young) genes and polymorphisms in TCF7L2"** was done by Dr.D.M.Mahesh, for the degree of DM in Endocrinology at Christian Medical College, Vellore.

This study was undertaken in the Department of Endocrinology, Diabetes and Metabolism, Christian Medical College, under my direct supervision, guidance and to my complete satisfaction, as a part of the requirement for the award of the degree DM in Endocrinology.

Guide:

Dr. Nihal Thomas,
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Professor &Head,
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Christian Medical College, Vellore – 632 004.

CERTIFICATE

This is to certify that this research work titled **"The Study of pregnant women with diabetes and screening for mutations related to common MODY (Maturity Onset Diabetes of the Young) genes and polymorphisms in TCF7L2"** was done by Dr.D.M. Mahesh, for the degree of DM in Endocrinology, Christian Medical College, Vellore under the guidance of Prof. Nihal Thomas, Professor and Head, Department of Endocrinology, Diabetes & Metabolism, Christian Medical College, as a part of the requirement for the award of the degree DM in Endocrinology.

Professor Alfred Job Daniel,
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Tamil Nadu

ACKNOWLEDGMENT

A thesis is much more than just a document. It is not just a piece of evidence that you have actually done something during the years of training. It is a reminder of the endless hours off writing it and messages that begin with “I am sorry…….”. But most of all, it is part of the hard work of many people who have put their energy selflessly for me to prepare a document to help convince a panel of experts that, I understood what was being done. It provides a strong foundation and stands as a gateway to future research. Though words will not be enough to express my gratitude, I would still like to thank all those people who made this dissertation a reality. I realize that it was, indeed, teamwork that got me there.

It is difficult to overstate my gratitude towards my mentor and guide Prof. Nihal Thomas. From the time I joined the department, I am eternally indebted to him for all the guidance and encouragement that made the journey of this research a challenge and joy. With his enthusiasm, hard work, out of the box thinking, ability to push you to your limits and get the best out of you, he has helped to make my tenure a success. The zeal and infinite energy he has for research is contagious and motivational for me. I am also indebted for the excellent example he has provided as a successful clinician and remarkable teacher.

I owe my most sincere gratitude to my friend and molecular scientist Aaron Chapla and his team viz., Denny, Johan, Manika, Papitha whose effort is the sole reason in the fructification of my research work. I shall always cherish the endless discussions with Aaron in the lab and helping me understand the jargons of molecular biology.

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In my daily work, I have been blessed with a friendly and cheerful group of fellow colleagues. In particular, I would like to thank Nitin, for his friendship and a supporting shoulder whenever I needed. I would like to thank all my dearest colleagues Shilpa, Anjali, Anulekha, Bidyut, Ron, Sahana, Riddhi, Felix, Srinath, Rita, Sharada, Sufiya, Veena and Padmanaban for all the times we were working together. I also want to express my sincere thanks to Mr. Mohan for the never ending and timely help he provides whenever needed. I'm thankful for the support I received throughout my tenure from Mrs. Ruth and the diabetes educators including all the members of the staff of ***"Endo-Family"***, our department.

Most importantly, I have no suitable word that can fully describe my gratitude for my wife Dr. Sneha Patil. This study is a result of her help and selfless love. It seems like she always knows what goes on in my mind and even without speaking, she has the right words to say. She has always encouraged me patiently with her support.

Last but not the least I wish to thank Almighty God, my parents, Mruthyunjaya and Swarnamba and my parents in law, J.M.Patil and Sashikala, who have always encouraged me to pursue my dream.

I also take this opportunity to thank all my patients and their family members whose contribution towards this work is invaluable, for which my mere expression of thanks likewise does not suffice.

Mahesh.

ANTIPLAGARISM

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ABBREVIATIONS	
ACOG	American College of Obstetricians and Gynecologists
ADA	American Diabetes Association
ADCY5	Adenylate Cyclase Type 5
BMI	Body Mass Index
CDK	Cyclin Dependent Kinase
CDKAL1	CDK5 regulatory subunit associated protein 1-like 1
CTLA	clathrin, light chain A
CTLA-4	Cytotoxic T-Lymphocyte Antigen 4 / CD152 (Cluster of differentiation 152)
DIPSI	Diabetes in Pregnancy Study Group of India
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
FTO	Fat mass and obesity associated (alpha-ketoglutarate-dependent dioxygenase)
GAD	Glutamic acid decarboxylase (GAD)
GDM	Gestational Diabetes mellitus
HbA1c	Glycated hemoglobin
HLA	Human Leucocyte Antigen
HPL/HCS	Human placental lactogen (hPL), also called human chorionic somatomammotropin (HCS)
ICA	Islet cell antibodies
IGF2BP2	Insulin-like growth factor 2 mRNA-binding protein 2
IGT / IFG	Impaired Glucose tolerance(IGT) / Impaired fasting glucose (IFG)
IRS-1	Insulin Receptor Substrate-1
KCNJ11	Potassium inwardly-rectifying channel, subfamily J, member 11
KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1
MODY	Maturity Onset Diabetes of the Young
MTNR1B	Melatonin receptor 1B
OGTT	oral glucose tolerance test
OVERT	Overt diabetes in pregnancy
PolyPhen-2	Polymorphism Phenotyping v2
PPARG	Peroxisome proliferator-activated receptor gamma (PPAR-γ)
PREGDM	Pregestational diabetes
SIFT	Sorting Intolerant From Tolerant
SNP	Single Nucleotide Polymorphism
T1D	Type 1 Diabetes mellitus
T2D	Type 2 Diabetes mellitus
TCF7L2	Transcription factor 7-like 2 (T-cell specific, HMG-box)
TNFα	Tumour Necrosis Factor - alpha
VNTR	Variable Number Tandem Repeat
WHO	World Health Organization

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September 17, 2012

Dr. D.M. Mahesh
PG Registrar
Department of Endocrinology
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Vellore 632 002

Sub: **FLUID Research grant project NEW PROPOSAL:**
The study of pregnant women with diabetes and screening for mutations related to common MODY (Maturity Onset Diabetes of the Young) genes and polymorphisms in TCF712.
Dr. D.M. Mahesh, Endocrinology, Dr. Nihal Thomas, Dr. Thomas V Paul, Dr. Simon Rajaratnam, Dr. Asha H.S, Dr. Nishanth Arulappan, Mr. Aaron Chapla, Endocrinology, Dr. Molly Jacob, Biochemistry, Dr. Jiji Mathew, Dr. Ruby Jose, Dr. Jessie Lionel, Dr. Annie Regi, Obstetrics and Gynaecology, Dr. K. Anil Kuruvilla, Neonatology, Dr. L. Jeyaseelan, Biostatistics.

Ref: IRB Min. No. 7998 dated 08.09.2012

Dear Dr. Mahesh,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "The study of pregnant women with diabetes and screening for mutations related to common MODY (Maturity Onset Diabetes of the Young) genes and polymorphisms in TCF712" on September 9, 2012.

The Committees reviewed the following documents:

1. Format for application to IRB submission
2. Patient Information Sheet and Informed Adult Consent Form (English, Tamil, Hindi and Telugu)
3. Cvs of Drs. Mahesh, Nihal Thomas, Thomas V Paul, Simon Rajaratnam, Asha H.S, Nishanth Arulappan, Mr. Aaron Chapla, Molly Jacob, Jiji Mathew, Ruby Jose, Jessie Lionel , K. Anil Kuruvilla, L. Jeyaseelan.
4. A CD containing documents 1 – 3

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The following Institutional Review Board (Research & Ethics Committee) members were present at the meeting held on September 9, 2012 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Other Affiliations
Mr. Sampath	BSc, BL	Advocate	External
Mrs. Ellen Ebenezer Benjamin	M.Sc. (Nursing), Ph.D.	Professor, Maternity Nursing, CMC.	
Mr. T.S. Ravikumar	MSC	Professor, Medical Surgical Nursing, CMC	
Mr. Harikrishnan	BL	Lawyer, Vellore	External
Mr. Joseph Devaraj	BSc, BD	Chaplain, CMC	
Dr. Priya Abraham	MBBS, MD, PhD	Professor, Virology, CMC	
Dr. Bobby John	MBBS, MD, DM, PhD, MAMS	Professor, Cardiology, CMC.	
Dr. Anil Kuruvilla	MBBS, MD, DCH	Professor, Neonatology, CMC.	
Dr. Simon Rajaratnam	MBBS, MD, DNB (Endo), MNAMS (Endo), PhD (Endo), FRACP	Professor, Endocrinology, CMC	
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC	
Dr. Benjamin Perakath	MBBS, MS, FRCS	Professor, Surgery (Colorectal), CMC.	
Dr. Paul Ravindran	PhD, Dip RP, FCCPM	Professor, Radiotherapy, CMC	
Dr. Sujith Chandy	MBBS, MD	Professor, Pharmacology & Clinical Pharmacology, CMC.	
Dr. Denny Fleming	BSc (Hons), PhD	Honorary Professor, Clinical Pharmacology, CMC.	

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We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any serious adverse events occurring in the course of the project, any changes in the protocol and the patient information/informed consent. And on completion of the study you are expected to submit a copy of the final report.

Yours sincerely

Dr. Alfred Job Daniel
Principal & Chairperson (Research Committee)
Institutional Review Board

Chairperson (Research Committee) &
Principal
Christian Medical College
Vellore - 632 002, Tamil Nadu, India

CC: Dr. Nihal Thomas, Professor, Department of Endocrinology, CMC

INTRODUCTION

Diabetes mellitus (DM) has evolved into a global epidemic and India has the second largest population with diabetes. With a prevalence of 9.09%, India currently harbours 65.1 of the 382 million people affected with diabetes(1). Along with the classical type 2 (T2D) and type 1 diabetes mellitus (T1D), it includes gestational diabetes mellitus (GDM), monogenic forms of diabetes and secondary diabetes. 'Gestational Diabetes Mellitus' has been defined as *"carbohydrate intolerance of any degree of severity with onset or first recognition during pregnancy"*(2). Around 7% of all pregnancies (1 to 14%, depending on the diagnostic tests employed and the population studied) are complicated by GDM, and hence resulting in approximately 200,000 cases annually(2). In India, the prevalence of GDM has varied from 3.8 to 21% in different parts of the country (3,4). In 2013, more than 21 million live births were affected by diabetes during pregnancy(1). Further, the prevalence of GDM corresponds to the prevalence of impaired glucose tolerance [IGT], in a non-pregnant adults within that population (5).

The complex interaction between the environment and the genetic makeup play a role in the pathophysiology of diabetes which may have its own peculiarities in relation to the origin of the disease in the Indian subcontinent(6). Besides standard environmental factor, maternal malnutrition and low birth weight may also have an impact(7). Several risk factors are associated with the development of GDM, the most common being obesity diagnosed before pregnancy. Classical type 1 and type 2 diabetes are considered to be polygenic(8), however monogenic forms of diabetes have been discovered, with specific genes being implicated in each case. Maturity-onset diabetes of the young (MODY) that may account for upto 2% of patients encompasses a monogenic form of diabetes, that is predominantly inherited in an autosomal dominant mode or may occasionally occur as a de novo mutation. MODY can result from mutations in at least one of the thirteen genes that have been reported till date(9). The clinical presentations

of MODY are heterogeneous, reflecting the many gene mutations involved, and the glucose dysregulation observed ranges from a relatively mild elevation in the fasting glucose to overt diabetes (10). An exact MODY prevalence within the general diabetes population has proven to be difficult to assess owing to the under recognition and lack of routinely available and affordable diagnostic tools. A further confounder is that the regional prevalence of specific mutations in MODY genes vary considerably (10). It is usually expected that among women screened for GDM, the prevalence of MODY will be higher, reflecting both the probability that those with undiagnosed MODY will screen positive and the proportionately lower prevalence of type 2 diabetes in women of this age group. The ongoing epidemic of obesity and diabetes has led to more T2D in women and hence the number of pregnant women with undiagnosed T2D has increased. Furthermore, not only are women diagnosed to have GDM but also their children are at an increased risk of future T2D. There has also been a trend towards a shift in the mean age of onset of diabetes (perceived as T2D) to a much younger age, that ranges between 25 to 34 years(11). Due to the overlap of clinical features with polygenic diabetes, this subset of patients are often misdiagnosed as T1D or T2D(10) and may receive inappropriate therapy (12,13). Therefore, distinguishing MODY from other common forms of diabetes and deciding as to who would benefit from genetic screening has been a major challenge(14). From the few population studies reported, mutations in the hepatocyte nuclear factor 1 alpha (HNF1A) gene (MODY 3) and glucokinase (GCK) gene (MODY 2) account for the majority of cases (15). However, except for four Indian studies (85-88) that had looked at specific genes implicated in MODY in a non GDM population, little is known about the genetic basis of GDM in India and its potential clinical significance. Identification of the possible underlying MODY genetic factors in GDM would enrich our knowledge on the pathophysiological mechanism of the disease and identification of susceptible individuals in their family,

which in turn, will enable preventive and therapeutic intervention for both the mother and the developing fetus. Due to the increased cost and limited availability of genetic diagnostic facilities in India, only few mutations or polymorphisms have been studied utilising the Sanger sequencing methodology in a small set of patients clinically diagnosed as MODY(18,19). However, with the advent of next-generation sequencing (NGS) technology, there has been a dramatic improvement in cost, speed and scalability of sequencing(20,21). Further, till date none of them have taken the whole genetic spectrum of MODY genes in pregnant women with diabetes into account and scanned for the mutations and polymorphisms which may be unique to this population. Therefore, we utilizing a semiconductor based second-generation sequencing platform(22) aimed to screen for mutations in a panel of ten MODY genes viz., HNF4A: Hepatocyte Nuclear factor 4 alpha, GCK, HNF1A, IPF1: Insulin promoter factor 1, HNF1B: Hepatocyte nuclear factor 1 beta, NEUROD1: Neurogenic differentiation factor 1, KLF11: Kruppel-like factor 11, CEL: Carboxyl ester lipase, PAX4: Paired box 4 and INS: Insulin gene in pregnant women with diabetes.

Genome wide association studies (GWAS) in T2D have shown that *TCF7L2 (*Transcription factor 7-like 2) variants represent one of the strongest genetic signals associated to an increased risk of T2D. It's been hypothesised that TCF7L2 play a pathogenic role through several mechanisms, including decreased beta-cell mass (23), impaired insulin processing or release (24), impaired GLP-1 signalling in beta cells (25)(26) and increased hepatic gluconeogenesis (27). The common variants of TCF7L2 have showed an association with diabetes mellitus in European (28) and Asian population(29)(30) {OR=1.3-1.9}. In this study we also assessed the associations of three *TCF7L2 single nucleotide polymorphisms* (rs7903146,rs12255372 and rs4506565) with gestational diabetes.

SECTION-I

1. Screening of pregnant women with diabetes for mutations in a panel of ten MODY genes {HNF1A, HNF4A, GCK, HNF1β, IPF1(PDX1), NEUROD1, KLF11, CEL, PAX4, and INS} by utilizing the second generation sequencing platform and genotype-phenotype correlation (GPC)

SECTION-II

2. To study the association of three common TCF7L2 polymorphisms (rs7903146, rs12255372 and rs4506565) in the occurrence of gestational diabetes.

DIABETES AND PREGNANCY

Pregnancy has been traditionally described as a transient excursion into the metabolic syndrome (31). During pregnancy, metabolic changes are necessary to ensure the growth and development of the foetus and in order to meet the demands of the pregnant mother. In addition, both are provided with adequate energy stores that are needed during labour and during the period of lactation. In general, during the latter half (end of second trimester) of the pregnancy, insulin sensitivity reduces and insulin secretion increases. Glucose seems to be the major substrate for the human foetus during pregnancy, and glucose metabolism has thus been the subject of most studies on metabolism in pregnancy. Pregnancy - related maternal insulin resistance benefits foetal growth, because a rise in post - prandial glucose concentration aids glucose transfer to the foetus, a process termed “facilitated anabolism” (32). Maternal to foetal glucose transfer in the fasting state is enhanced by maternal lipolysis, which occurs in late pregnancy, with free fatty acids becoming the main maternal fuel substrate and diversion of glucose to the foetus. The ability of insulin to suppress lipolysis (via inhibition of hormone - sensitive lipase in adipose tissue) is severely impaired in late pregnancy, when maternal free fatty acid release and fatty acid oxidation are increased in parallel with reduced carbohydrate oxidation. This process of enhanced lipolysis has been termed “accelerated starvation” and is attributed to the actions of human placental growth hormone and other placental hormones (33). These metabolic changes facilitate the transfer of glucose and amino acids to the foetus. An increase in hepatic glucose output in late pregnancy, owing to hepatic insulin resistance, ensures that maternal glucose is available to the foetus between meals (34). Hence, the relative insulin resistance in pregnancy stabilizes glucose input to the foetus and the role of placental hormones seems to be crucial (Figure.1)

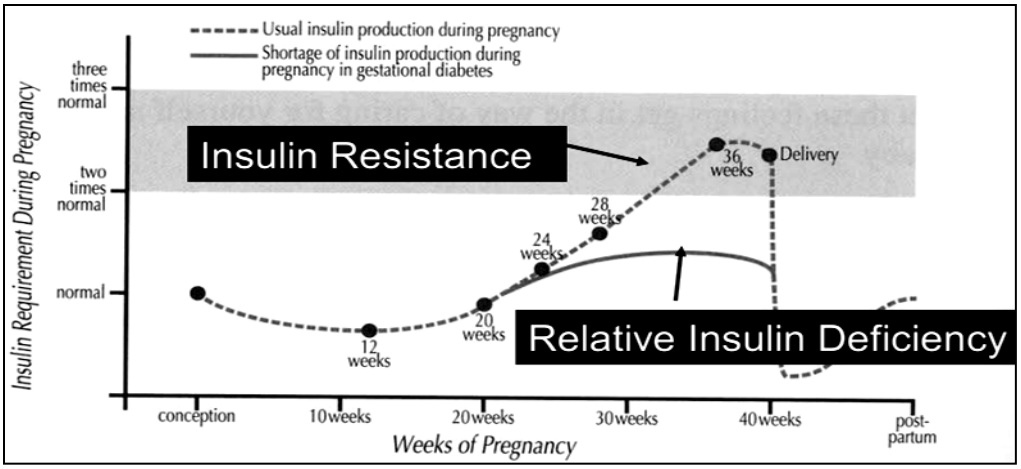


Figure.1.The relative insulin deficiency in the presence of insulin resistance that sets in at around 20 weeks results in hyperglycemia in pregnancy. Adapted from:http://www.idcpublishing.com/Gestational-Diabetes.

Gestational Diabetes Mellitus: Pathophysiology

Pregnancy is characterized by progressive insulin resistance that begins near second half (20th week) and progresses through the third trimester (Fig.1). In late pregnancy, insulin sensitivity falls by around 50% (35). Two main factors to insulin resistance are increased maternal adiposity and the insulin desensitizing effects of placental hormones. The fact that insulin resistance rapidly decreases after birth suggests that the main contributors are hormones secreted by the placental. Placental hormones such as progesterone, prolactin, cortisol and human placental lactogen released in the second half, contribute to decreased insulin action in pregnancy. The placental human chorionic somatomammotropin (HCS, formerly called human placental lactogen) stimulates secretion of insulin by foetal pancreas and inhibits peripheral uptake of glucose in the mother (36). As the pregnancy progresses, the size of the placenta increases, so does the production of the hormones, leading to an increase in insulin-resistant state. In non diabetic pregnant women, this is associated with b-cell hypertrophy and hyperplasia resulting in increased the first- and second phase insulin responses that compensate for the reduction in insulin sensitivity (37). It has been suggested that women who develop GDM may also have an underlying deficit in this additional insulin secretion [beta cell

dysfunction]. In pregnant women with abnormal glucose intolerance, the insulin resistance of pregnancy is not adequately compensated for, resulting in carbohydrate or glucose intolerance (36). Beta-cell dysfunction in women diagnosed with GDM may fall into one of three major categories: *1*) occurring on a background of insulin resistance (as is most common) *2*) monogenic, or *3*) autoimmune (38). The loss of the first-phase insulin response leads to postprandial hyperglycemia, whereas inadequate suppression of hepatic glucose production is responsible for fasting hyperglycemia. In the lean pregnant woman with normal glucose tolerance, there is a significant 30% increase in basal hepatic glucose production by the third trimester of pregnancy(39). The relationship between insulin sensitivity and insulin response has been characterized by Bergman and colleagues as a hyperbolic curve or, when multiplied, as the *disposition index*(40). A curve that is "shifted to the left" can be plotted for individuals who go on to develop GDM (Figure. 2). Whether the insulin resistance precedes the beta cell defect or as to whether they occur concomitantly is not known with certainty(41). However, Buchanan proposed that insulin resistance precedes the beta cell dysfunction in susceptible individuals (38). The increased risk of type 2 diabetes in women who formerly had GDM may be a function of decreasing insulin sensitivity (i.e., worsening insulin resistance) exacerbated by increasing age, adiposity, and the inability of the beta cells to fully compensate(42). Because insulin does not cross the placenta, the foetus is exposed to the maternal hyperglycemia.

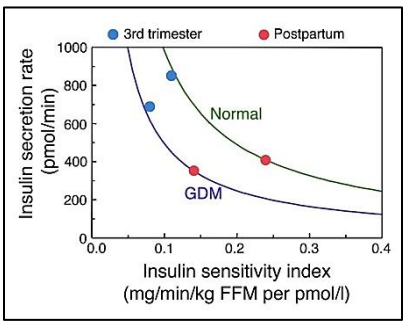


Figure.2: Insulin sensitivity-secretion relationships in women with GDM and normal women during the third trimester and remote from pregnancy. Values were measured at the end of 3-hour hyperglycemic clamps (plasma glucose, about 180 mg/dl) (22). Pre-hepatic insulin secretion rates were calculated from steady-state plasma insulin and C-peptide levels. Insulin sensitivity index was calculated as steady-state glucose infusion rate divided by steady-state plasma insulin concentration(41). (FFM, fat-free mass, Figure adapted from Buchanan TA et al, J. Clin. Endocrinol. Metab.2001;86:989-993. Copyright 2001.Endocrine Society).

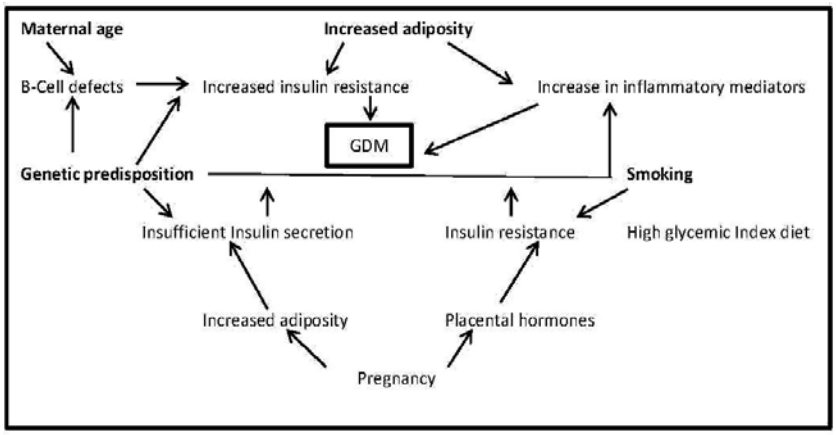


Figure 3: Mechanism of gestational diabetes mellitus
{adapted from Hezelgrave et al. Expert Rev EndocrinolMetab. 2012;7(6):669-676 }

Mechanisms of Insulin Resistance:

The mechanisms related to the changes in insulin resistance during pregnancy are better characterized because of research in the past decade. The insulin resistance of pregnancy is almost completely reversed shortly after delivery consistent with the clinically marked decrease in insulin requirements(43). The placental mediators of insulin resistance in late pregnancy have been ascribed to alterations in maternal cortisol concentrations and placenta-derived hormones such as human placental lactogen (HPL), progesterone, and estrogen(44). Kirwan et al. reported that circulating tumor necrosis factor-α (TNF-α) concentrations had an inverse correlation with insulin sensitivity as estimated from clamp studies(45). Among leptin, HPL, cortisol, human chorionic gonadotropin, estradiol, progesterone, and prolactin, TNF-α was the only significant predictor of the changes in insulin sensitivity from the pregravid period through late gestation. TNF-α and other cytokines are produced by the placenta, and 95% of these molecules are transported to maternal rather than foetal circulations(45). Other factors, such as circulating free fatty acids, may contribute to the insulin resistance of pregnancy(32,40). Studies in human adipose tissue and skeletal muscle have demonstrated defects in the post-receptor insulin-signaling cascade during pregnancy. Friedman et al showed that women in late pregnancy have reduced insulin receptor

substrate-1 (IRS-1) concentrations compared with those of matched nonpregnant women(47). Down regulation of the IRS-1 protein closely parallels insulin's decreased ability to induce additional steps in the insulin signalling cascade that result in the glucose transporter (GLUT-4) arriving at the cell surface to allow glucose to enter the cell. Down regulation of IRS-1 closely parallels the decreased ability of insulin to stimulate 2-deoxyglucose uptake in vitro in pregnant skeletal muscle. During late pregnancy in women with GDM, in addition to decreased IRS-1 concentrations, the insulin receptor-β (i.e., component of the insulin receptor within the cell rather than on the cell surface) has a decreased ability to undergo tyrosine phosphorylation (47). This is an important step in the action of insulin after it has bound to the insulin receptor on the cell surface. This additional defect in the insulin-signaling cascade is not found in pregnant or nonpregnant women with normal glucose tolerance and results in a 25% lower glucose transport activity. TNF-α also acts by means of a serine/threonine kinase, thereby inhibiting IRS-1 and tyrosine phosphorylation of the insulin receptor (48). These post-receptor defects may contribute in part to the pathogenesis of GDM and an increased risk for type 2 diabetes in later life.

Link to diabetes after pregnancy.

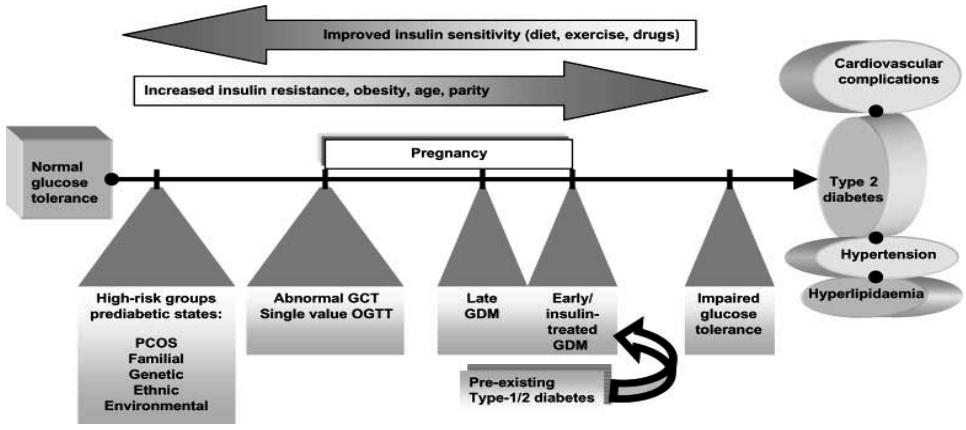


Figure 4: Suggested insulin resistance pathway. The progression from a normal glucose tolerance state to overt Type 2 diabetes may be accelerated by factors that increase insulin resistance and attenuated by life-style modifications and insulin-sensitizing drugs (such as metformin). Early onset of GDM, in the first half of pregnancy, and the need for insulin treatment—probably the result of a higher insulin-resistant state—may offer a greater risk of future development of Type 2 diabetes(49). (Adapted from Ben-Haroush et al.Diabetic Medicine 2003,21:103–113)

The hyperglycemia of GDM is detected at a single point in a women's life. If the plasma glucose levels are not in that of the diabetic range, GDM could represent glucose intolerance that is limited to pregnancy. It may also be is at a stage in the progression to diabetes or is chronic but stable. Long-term follow-up studies, reviewed by Kim et al. (50), reveal that most (ranging from 2.6% to over 70% in 6 weeks to 28 years postpartum), but not all, women with GDM do progress to diabetes after pregnancy. Only around10% of women with GDM have diabetes soon after delivery(51). Incident cases appear to occur at a relatively constant rate during the first 10 years (50). Outside of pregnancy, three general settings are recognized — through classification as distinct forms of diabetes— as separate forms of β cell dysfunction viz., (a) autoimmune; (b) monogenic; and (c) occurring on a background of insulin resistance (32). There is enough evidence that β cell dysfunction in GDM can occur in all the 3 main categories, a fact that is not surprising given that the detection of GDM is in essence, a population screening for increased glucose levels among women in child bearing age. Most studies on risk factors for the ocurance of diabetes after GDM do not distinguish among the possible subtypes of GDM and diabetes discussed above. The studies have identified risk factors, such as obesity, weight gain and increasing age, that are known to be associated with T2D (Figure.4). Longitudinal studies of the pathophysiology of diabetes that develops after GDM are well documented in Hispanic women(52)(53). The findings suggest a progressive decline in insulin secretion as a result of high insulin secretory demands imposed by insulin resistance. Lean patients are more likely to be insulin sensitive than overweight or obese people, therefore autoimmune or monogenic forms of diabetes should be considered in those women, who can progress to overt diabetes soon after pregnancy. Around 4.6% of the patients with GDM have been reported to develop type 1 diabetes during a 6 years of follow-up period (54). About 66% of these women had islet cell antibodies (ICAs), whereas 56% tested positive for autoantibodies

to Glutamic acid decarboxylase (GAD) (55). Also, mutations that cause several types of maturity- onset diabetes of the young (MODY) have been found to account for < 10% of GDM cases (56).

Maternal hyperglycemia in the first few weeks of pregnancy increases the risk of foetal malformations, spontaneous abortions, and perinatal mortality. Ideal preconception blood glucose levels have not been definitively established, and the exact degree of risk of a congenital anomaly for a given HbA1c (Glycated haemoglobin) is not precisely known. It has been reported that the risk progressively rises in concert with the degree of periconceptional HbA1c elevation, although an increased risk compared with the general childbearing population has been observed with an HbA1c as low as 6.4%(57). It has, however, also been reported that there is a stable degree of anomaly risk of 3.9% to 5.0% with a periconceptional HbA1c of up to 10.4%, with this risk then climbing to 10.9% if the HbA1C is 10.4% or higher(58). At around 11th or 12th week of gestation, the foetal pancreas is able to respond to this hyperglycemia. Thus, the foetus becomes hyperinsulinemic and in turn promotes growth and subsequent macrosomia(57).

Glucose levels in the diabetic range [overt diabetes] is a late consequence of the decline in insulin secretion(59). This loss can be slowed or halted through the treatment of insulin resistance in order to decrease high insulin secretory demands. A recent audit(59) identified 1579 women with GDM and 254 with overt diabetes in pregnancy. Women with overt diabetes in pregnancy were diagnosed in the first half of pregnancy, had a higher pre-pregnancy Body Mass Index (BMI), higher antenatal HbA1c, fasting and 2-h post meal glucose levels along with an increase in insulin use and dosage requirements than those with gestational diabetes. Overt diabetes in pregnancy was associated with a higher rate of macrosomia, neonatal hypoglycemia and shoulder dystocia. Of the subjects with overt diabetes in pregnancy (n=133) who had a follow-up

oral glucose tolerance test (OGTT) at 6-8 weeks post-partum, 21% had diabetes, 37.6% had impaired fasting glucose or impaired glucose tolerance, whilst 41.4% returned to normal glucose tolerance (59). In a similar study 1267 women had gestational diabetes and 348 had overt diabetes in pregnancy. Pregestational body mass index was higher and gestational age at diagnosis was earlier in overt diabetes than in gestational diabetes. HbA1c and glucose levels on 75-g oral glucose tolerance test and prevalence of retinopathy (1.2% vs. 0%, P<0.05) and pregnancy-induced hypertension (10.1% vs. 6.1%, P<0.05) were higher in overt diabetes than in gestational diabetes(60).

Genetics and Diabetes

Diabetes mellitus is a group of disorders who share the metabolic phenotype of persistent hyperglycemia. The T1D and T2D are the two most common forms. Both are arrtributed to a combination of genetic and environmental risk factors. However, other rare forms of diabetes like maturity onset diabetes in the young (MODY), and those due to mutations in mitochondrial DNA that are directly inherited. Further, two major paradigms include the genetic risk variants and behavioural /environmental factors jointly underlie the development of T2D and related coronary artery disease, diabetic nephropathy, and diabetic retinopathy.

Genetics and Type 1 Diabetes

The risk of developing T1D in first degree relatives is higher than unrelated subjects in the general population (~6% vs. <1%, respectively) (61). At present, genetic susceptibility to T1D has been linked to more than 20 regions of the genome. However, none of the candidates genes have a significantly higher influence on T1D risk than that conferred by genes in the HLA region of chromosome 6, known to be involved in immune response. The HLA (Human leukocyte antigen) class II genes (i.e., HLA-DR, DQ, DP) are most strongly associated with the disease.

Type 1 Diabetes Mellitus(T1D) : The HLA class II genes contribute to about 40-50% of the heritable risk for T1D (62). When evaluated as haplotypes, DQA1*0501-DQB1*0201 and DQA1*0301-DQB1*0302 are most strongly associated T1D in Caucasian populations. Other reported high risk haplotypes for T1D include DRB1*07-DQA1*0301-DQB1*0201 among African Americans, DRB1*09-DQA1*0301-DQB1*0303 among Japanese, and DRB1*04-DQA1*0401-DQB1*0302 among Chinese. Further, the DRB1*15-DQA1*0602-DQB1*0102 is protective and associated with a reduced risk of T1D in most populations(62). People with two high risk DRB1-DQA1-DQB1 haplotypes have a significantly higher T1D risk than individuals with no high risk haplotype. In terms of absolute risk, Caucasian individuals with two susceptibility haplotypes have an approximately 6% chance of developing T1D through age 35 years. However, this figure is substantially lower in populations where T1D is rare (i.e., < 1% among Asians). Two other genes *INS* and *CTLA-4,*are now known to influence T1D risk (63) (Table-1).

Table-1: T1D susceptibility gene and estimated relative risk (RR)

T1D Susceptibility Genes	Locus	Variant	Estimated RR
HLA-DQB1	6p21.3	*0201 & *0302	3 – 45
INS	11p15. 5	Class I	1 – 2
CTLA4	2q31-35	Thr17Ala	1 – 2

Genetics, T2D & GDM

Recently, significant advance has been made in identifying susceptible genes involved in T2D through genome-wide association strategy (GWAS) (64)(65). Consequently, a number of novel genetic variants (PPARG, KCNJ11, IGF2BP2, KCNQ1, TCF7L2, CDKAL1, and MTNR1B) are reported to increase the risk of T2D in many studies. Functional studies have revealed that these new diabetogenic genes are linked to impaired b-cell function (CDKAL1, IGF2BP2, KCNQ1, KCNJ11, MTNR1B), insulin resistance (PPARG, TCF7L2), and abnormal utilization of glucose (GCK) (58)(66). Although its exact reason is unknown, recent evidence recognizes GDM as a quintessential multifactorial disease in which genetic variants interact with environmental

triggers (50)(67)(68). Cho *et al.* examined 18 single nucleotide polymorphisms (SNPs) in nine T2DM susceptibility loci and found that two loci, *CDKAL1* and *HNF1B* (encoding a member of the homeodomain-containing family of transcription factors), showed marginal association with GDM. *CDKAL1*, encoding a protein of unknown function but having high sequence homology with proteins regulating cyclin-dependent kinase involved in cell cycle regulation, showed strong association with GDM(69). Interestingly, the odds ratios for GDM were slightly higher than typically observed for T2DM (about 1.5 vs 1.2). The studies by Cho *et al.* (68) and Lauenborg *et al* (66)suggest that at a minimum, there is some overlap in genetic susceptibility to GDM and T2DM. This overlap in genetic susceptibility may partly explain the increased risk for T2DM in women with previous GDM (70). The BetaGene study, a family-based genetic study involving GDM has shown that these associations are modified by other factors, such as adiposity or other gene variants(70). For example, variation in TCF7L2 and OGTT-based insulin secretion (β-cell function) is modified by total body fat (70)(71)(72). This may represent potential gene-gene interactions, as GWA studies have detected variants underlying susceptibility to obesity and contributing to variation in obesity-related traits(71-73). The rs9939609 variant in the *FTO* locus is associated with measures of adiposity and metabolic consequences in South Indians with an enhanced effect associated with urban living(73). Alternatively, body fat could also reflect the net caloric balance between dietary intake (quantity and composition) versus physical activity (type and intensity). Further, the 'birth-weight-lowering' variant of ADCY5 was found to be associated with glucose intolerance in early adulthood which argues for a common genetic cause of low birth weight and risk of type 2 diabetes(74). The effect sizes of loci identified so far have been, for the most part, extremely small (OR < 1.5) from a clinical or epidemiological perspective(70). Clearly, additional studies will be required to better understand this complex interaction.

MUTATIONS & POLYMORPHISM (75)

A gene mutation is a permanent change in the DNA sequence that makes up a gene. Gene mutations have varying effects on health, depending on where they occur and whether they alter the function of essential proteins. Gene mutations occur in two ways: they can be inherited from a parent or acquired during a person's lifetime. Mutations that are passed from parent to child are called hereditary mutations or germ line mutations (because they are present in the egg and sperm cells, which are also called germ cells). This type of mutation is present throughout a person's life in virtually every cell in the body. Mutations that occur only in an egg or sperm cell, or those that occur just after fertilization, are called new (de novo) mutations. De novo mutations may explain genetic disorders in which an affected child has a mutation in every cell, but has no family history of the disorder. Acquired (or somatic) mutations occur in the DNA of individual cells at some time during a person's life. These changes can be caused by environmental factors such as ultraviolet radiation or can occur if an error occurs as DNA copies itself during cell division. Acquired mutations in somatic cells (cells other than sperm and egg cells) cannot be passed on to the next generation(75).

Some genetic changes are very rare; others are common in the population. Genetic changes that occur in more than 1% of the population are called polymorphisms(75). They are common enough to be considered a normal variation in the DNA. Polymorphisms are responsible for many of the normal differences between people such as eye colour, hair colour, and blood type. Although most polymorphisms have no negative effects on a person's health, some of these variations may influence the risk of developing certain disorders.

The DNA sequence of a gene can be altered in a number of ways. The types of mutations are illustrated in figure 4.

The types of mutations are:

Missense mutation

This type of mutation is a change in one DNA base pair that results in the substitution of one amino acid for another in the protein made by a gene.

Nonsense mutation

A nonsense mutation is also a change in one DNA base pair. Instead of substituting one amino acid for another, however, the altered DNA sequence prematurely signals the cell to stop building a protein. This type of mutation results in a shortened protein that may function improperly or not at all.

Insertion

An insertion changes the number of DNA bases in a gene by adding a piece of DNA. As a result, the protein made by the gene may not function properly.

Deletion

A deletion changes the number of DNA bases by removing a piece of DNA. Small deletions may remove one or a few base pairs within a gene, while larger deletions can remove an entire gene or several neighboring genes. The deleted DNA may alter the function of the resulting protein(s).

Duplication

A duplication consists of a piece of DNA that is abnormally copied one or more times. This type of mutation may alter the function of the resulting protein.

Frameshift mutation

This type of mutation occurs when the addition or loss of DNA bases changes a gene's reading frame. A reading frame consists of groups of 3 bases that each code for one amino acid. A frameshift mutation shifts the grouping of these bases and changes the code for amino acids. The resulting protein is usually nonfunctional. Insertions, deletions, and duplications can all be frameshift mutations.

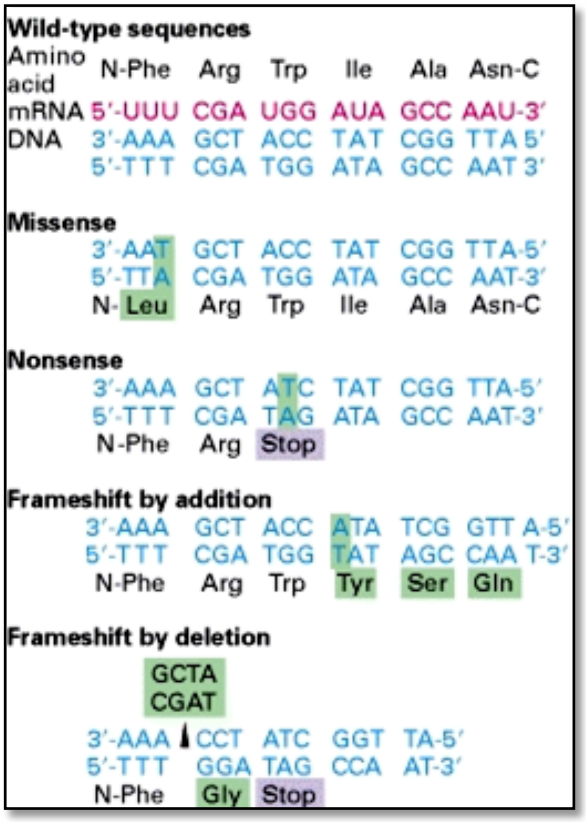


Figure.4: Illustration of types of common mutations: A mutation involving a change in a single base pair or a deletion of a few base pairs generally affects the function of a single gene. A wild-type peptide sequence and the mRNA and DNA encoding it are shown at the top. Altered nucleotides and amino acid residues are highlighted in green. Point mutations, which involve alteration in a single base pair and small deletions directly, affect the function of only one gene. Missense mutations lead to a change in a single amino acid in the encoded protein. In a nonsense mutation, a nucleotide base change leads to the formation of a stop codon (purple). This results in premature termination of translation, thereby generating a truncated protein. Frameshift mutations involve the addition or deletion of any number of nucleotides that is not a multiple of three, causing a change in the reading frame. Consequently, completely unrelated amino acid residues are incorporated into the protein prior to encountering a stop codon (75).
(Adapted from:
http://www.ncbi.nlm.nih.gov/books/NBK21578)

MONOGENIC DIABETES:

Monogenic diabetes is a clinically, metabolically, and genetically heterogeneous group of diabetes that results from beta-cell dysfunction due to mutations in a single gene. It includes the Maturity Onset Diabetes of the Young (hereafter referred to as MODY), neonatal diabetes (transient and permanent), mitochondrial diabetes, and other syndromic forms of diabetes. MODY occurs in families in whom there is an underlying mutation causing a defect in either β cell development or functioning. These mutations that drive the diabetic phenotype in concert with modifier genetic and/or environmental factors may contribute to clinical variability seen in familial young onset diabetes. The nonsense (truncating) or missense mutations that severely affects the protein function or occurrence of two or more co-segregating mutations, result in varying degrees of β cell developmental or functional defect. Therefore, based on type of mutation and severity of disease, these subjects may have as mild, moderate and severe forms of diabetes.

MATURITY ONSET DIABETES OF THE YOUNG

The term MODY (Maturity Onset Diabetes of the Young), first recognized by Tattersall refers to the most common form of monogenic diabetes, which is predominantly non-insulin dependent(76).

The classical(77) clinical features for diagnosing MODY include:

1. Early onset of Diabetes (<25 years)
2. Early onset Diabetes in at least 2, or ideally 3 family members
3. Autosomal dominant mode of inheritance
4. Non-insulin dependence (not requiring insulin even after 5 years of diagnosis (i.e., outside the ‘honeymoon’ phase in Type 1 diabetics)

Due to overlap of clinical features it can be falsely diagnosed as either T1D or T2D. Patients who are labelled as either T1D or T2D, but lacking the characteristic clinical features are possible MODY patients.

In those labelled as Type 1 diabetic patients, MODY is suspected when there is:

1. Strong family history
2. Evidence of non-Insulin dependence
3. Absence of GAD antibodies against pancreatic antigens
4. Detectable C-peptide outside the 'honeymoon period' (period of up to 5 years following diagnosis of Type 1 Diabetes, where there is still some endogenous Insulin secretion)

In those labelled as Type 2 diabetic patients, MODY is suspected when there is:

1) Younger age (less than 35 years) of onset
2) Lack of obesity
3) Lack of signs of Insulin resistance (acanthosis nigricans, polycystic ovaries)
4) Elevated or normal HDL levels, with reduced or normal triglyceride levels

Though Murphy et al, consider that the term MODY was outdated(78), reviewing the historical context enables one to understand why such a definition was adopted in the first place(79).

Table-2: The following table illustrates the differences between the three groups.

Parameters	Type 1 DM	Type 2 DM	MODY
Frequency	Common	Increasing	2 – 5% of non - insulin dependent Diabetics
Genetics	Polygenic	Polygenic	Autosomal Dominant
Family History	<15%	>50%	100%
Ethnicity	Different races	Asians, Polynesians, Indigenous Australians	Different races
Age of onset	Throughout Childhood	Post-Puberty	<25 yrs
Severity of onset	Acute and severe	Mild	Mild/Asymptomatic
Ketosis / DKA	Common	Uncommon	Rare
Obesity	+/−	>90%	+/−
Acanthosis Nigricans	Absent	Common	Absent
Metabolic Syndrome	Absent	Common	Absent
Auto-immunity	Positive	Negative	Negative
Pathophysiology	β Cell destruction	Insulin resistance and relative insulinopaenia	β Cell dysfunction

Ref: Investigating Maturity Onset Diabetes of the Young. ClinBiochem Rev.2009, 30 (ii) : 67-74

GENETIC BASIS OF MODY

The following table below shows displays profile of the genes implicated in MODY.

Table-3: Profile of the genes implicated in MODY

MODY type	Gene locus	Chromosome	OMIM	Prevalence (%)	Phenotypic features
MODY 1 (1991)	HNF 4α	20q13	125850	5-10	Progressive insulin secretory defect. Foetalmacrosomia, transient Neonatal hypoglycemia.
MODY 2 (1993)	GCK	7p13	125851	7-41	Stable mild fasting hyperglycemia (110-145mg/dl). Asymptomatic/ abnormal GTT/ Gestational DM, LBW. Complications rare
MODY 3 (1996)	HNF 1α	12q24	600496	11-63	Progressive insulin secretory defect. Renal glycosuria Low hsCRP, Increased HDL-cholesterol
MODY 4 (1997)	PDX 1/ IPF1	13q12	606392	2	Very rare. Associated with pancreatic agensis in homozygotes and occasionally in heterozygotes.
MODY 5 (1997)	HNF 1β	17q12	137920	2	Progressive diabetes, cystic renal disease (RCAD), genitourinary anomalies, pancreatic atrophy, exocrine dysfunction, hyperuricemia, gout, abnormal LFT
MODY 6 (1999)	NeuroD1	2q31	606394	1	Very rare: 5 families reported.
MODY 7 (2005)	KLF11	2p25	610508	<1	Rare
MODY 8 (2006)	CEL	9q34	609812	<1	Exocrine pancreatic dysfunction. Rare with five families reported.
MODY 9	Pax4	7q32	612225	<1	Rare
MODY 10	INS	11p15.5	613370	<1	Usually associated with neonatal diabetes. Rare < 1% cases.
MODY 11	BLK	8p23.1	613375	<1	Mutated B-lymphocyte tyrosin kinase, which is also present in pancreatic islet cells. Very rare.
MODY 12	ABCC8	11p15.1	606176	< 1	MODY & some forms of neonatal diabetes Tend to respond to sulfonylureas.
MODY 13	KCNJ11	11p15.1	601410	< 1	MODY & Some forms of neonatal-onset diabetes Tend to respond to sulfonylureas.
MODY X	Unknown	-	-	-	.-

[HNF4A: Hepatocyte Nuclear factor 4 alpha, GCK: Glucokinase, HNF1A: Hepatocyte nuclear factor 1 alpha, PDX1 or IPF1: Insulin promoter factor 1, HNF1B: Hepatocyte nuclear factor 1 beta, NEUROD1: Neurogenic differentiation factor 1, KLF11: Kruppel-like factor 11, CEL: Carboxyl ester lipase, PAX4: Paired box 4, INS: Insulin. BLK: tyrosine kinase, B-lymphocyte specific , ABCC8: ATP-binding cassette transporter sub-family C member 8, KCNJ11: Potassium channel(K+), inwardly rectifying, subfamily J, member 11.]

In the early 1960s-70s, MODY was diagnosed purely based on clinical features. During that period, studies from Europe showed that the prevalence of MODY was around 1-2% among the diabetic population (77). Though several genes have been implicated in monogenic diabetes the most common amongst them are HNF4α, HNF1α, and GCK. Hattersley et al, also suggest that the term 'MODY' is no longer relevant, and propose that monogenic diabetes (of which MODY is a subgroup) can be classified into 4 broad phenotypic categories:

1. Diabetes diagnosed before 6 months of age
2. Familial mild fasting hyperglycemia
3. Familial young onset diabetes (previously MODY)
4. Diabetes with extra-pancreatic features

GESTATIONAL DIABETES & COMMON MODY GENE MUTATIONS

Various genetic mutations predispose a pregnant woman to develop gestational diabetes(80). The importance of monogenic DM is due to the 50% risk of inheritance in offspring of affected subjects, the particular response of some types to sulphonyl-urea treatment(81), potential implications for treatment during pregnancy, comorbidities in specific types (HNF- 1β), and the insight provided for understanding gestational and Type 2 DM (78,82,83). In relation to the understanding of the genetics of common forms of DM, missense single nucleotide polymorphisms within MODY genes seem to be associated with common Type 2 DM(84,85).

PREVALENCE OF MODY IN PATIENTS WITH GESTATIONAL DIABETES MELLITUS

Studies examining the prevalence of mutation GCK gene (56,86–93) are characterized by being restricted to subgroups of women with GDM in order to increase the yield of the test.

Table-4: The table below highlights the details of the study.

MODY gene	Reference	No. of subjects studied	Selection criteria.	Prevalence (%)
GCK	Zoualli 1993(86)	17	- None	5
	Stoffel 1993(87)	40	- DM in first degree relatives	5
	Chiu 1994(88)	45	- Black ethnicity- No DM postpartum	0
	Saker 1996 (89)	50	-Postpartum FPG 100 - 180 mg/dl (5.5-10.0 mmol/l)	6
	Allan 1997(90)	50	- None	0
	Ellard 2000 (91)	15	- DM, GDM, or FPG >5.5 mmol/L in a in first degree relatives. Insulin during pregnancy & only diet after delivery with persisting fasting hyperglycemia outside pregnancy.	80
	Kousta 2001(92)	17	- Fasting hyperglycemia pregnancy & postpartum 99 - 144 mg/dl.	12
	Weng 2002(56)	66	- positive family history	5
	Zurawek 2007 (93)	119	*At least 3 of the following: Age <35years Prepregnancy BMI <25- Δ glucose OGTT< 83 mg/dl (4.6 mmol/l)- T2DM or GDM in FDR	2.5
HNF-1α	Weng 2002 (56)	66	- positive family history	1
	Zurawek 2007(93)	119	*As before	0
HNF- 4α	Weng 2002(56)	66	- positive family history	1
PDX1	Weng 2002(56)	66	- positive family history	1
NEUROD1	Sagen 2005(94)	51	- none	0

FDR: first degree relative; FPG: fasting plasma glucose; DM: Diabetes mellitus; GDM: gestational diabetes mellitus; OGTT: oral glucose tolerance test; BMI: body mass index; T2DM: Type 2 diabetes mellitus.

MONOGENIC DIABETES DUE TO MUTATIONS IN THE GLUCOKINASE (GCK) GENE:

Mutations in the GCK gene usually present as mild fasting hyperglycemia. Outside pregnancy, the following features have been identified as suggesting a GCK mutation and encouraging genetic testing for it: (95)

- Fasting hyperglycemia ≥ 100mg/dl, persistent and stable
- HbA1c typically just above the upper limit of normal
- In an oral glucose tolerance test the increment [(2 h glucose) − (fasting glucose)]is small, usually <80mg/dl
- Parents may have 'type 2 DM' with no complications or may not be diabetic

In addition patients with DM due a mutated GCK gene typically do not have signs of insulin resistance (78). If it has not been diagnosed before, the diagnosis will probably be made during gestation because the physiological resistance to insulin during the second half of pregnancy will uncover the defect in beta cell function. Depending on the criteria used(87,91,93), the prevalence is in the range of 0 (88,90) to 12(92) % with the exception of the study using more restrictive criteria(91) where the prevalence was 80%. In a study in the Polish population(30) it was shown that the frequency of Glucokinase gene mutation is 6.7% of gestational diabetic women and 17.8% of new onset or persistent diabetes recognized in the 5 years period after pregnancy could be a result of this mutation. The study also suggests that c.1253+8 C-->T polymorphism in intron 9 of glucokinase gene could have a role in predisposition to T2D in women with gestational diabetes.

MUTATIONS IN THE GCK GENE &FOETAL IMPACT

In the DM and pregnancy, Pedersen's hypothesis has been crucial to understanding foetal growth (97): The foetus of a woman with poorly regulated DM during pregnancy, is exposed to a higher than normal glucose load and responds with an increased insulin secretion which in turn leads to increased foetal growth. As GCK acts as the beta cell glucose sensor(98),foetuses that carry a GCK mutation are not able to mount an appropriate insulin response to the prevailing glucose levels and do not display the foetal growth that would be expected from maternal glycemia: non-affected foetuses of a GCK-deficient mother present with a high birth weight (BW), GCK-deficient

foetuses of a non-affected mother present with a low BW, whereas GCK-deficient foetuses of a GCK-deficient mother present with an essentially normal BW. In summary, harboring a GCK mutation implies a BW reduction of more than 500 g. In pregnancies of GCK-deficient foetuses, placentas have a reduced size (99), which clearly illustrate that foetal insulin is important for placental growth; on the other hand the reduced placental size could contribute to the reduced foetal growth. There are many studies linking foetal exposure to hyperglycemia with a higher and earlier rate of abnormal glucose tolerance, Type 2 DM or GDM (100). However in subjects with GCK-deficiency, no(101) or few (102) differences were detected in weight, height, or the secretion or insulin sensitivity in adulthood.

TREATMENT

In the summary and recommendations of the 5th International Workshop-Conference on GDM(103) it is acknowledged that after one randomized controlled trial during pregnancy (104) and several supporting observational studies, glyburide (glibenclamide) can be considered as a useful adjunct to medical nutritional therapy/ physical activity regimens when additional therapy is needed to maintain target glucose levels. Glyburide has not been tested in pregnant women with a GCK mutation but the fact that sulfonylureas increase GCK expression in vitro(105) and that a patient with permanent neonatal DM secondary to a homozygous GCK mutations displayed a partial response to treatment with sulfonylureas (106), would open the possibility that pregnant patients with a GCK mutation are treated with glyburide. However this would not include treatment during the first trimester in women with pre-existing diabetes (107).

During pregnancy it is very likely that women with DM due to a GCK mutation will present with raised blood glucose values usually prompting pharmacological treatment. However, in view of the opposing effects of maternal hyperglycemia and the presence of a foetal GCK mutation (101,108–112), maternal treatment should take into account the

foetal genotype. The problem is that the foetal genotype is not known and it does not seem justifiable to use a chorionic biopsy/ amniocentesis for this purpose.

MUTATIONS IN THE HNF-1α GENE:

Monogenic DM due to mutations in the gene transcription factor HNF-1α is the most common cause of MODY diabetes in most populations investigated and accounts for 1–2% of all diabetes (113). Progressive deterioration of insulin secretion does occur, and often requires pharmacological treatment and patients present with late diabetic complications, especially micro-angiopathic ones.

PREVALENCE OF HNF-1α MUTATIONS IN WOMEN WITH GDM

Only two studies have addressed the prevalence of HNF-1α mutations in women with GDM (56,93),both of them applying selection criteria and the rates being 0 and 1%.

FOETAL IMPACT

Contrary to other forms of monogenic DM, a foetus carrying a mutation in HNF-1α does not display differences in BW. Pearson et al (114), studied 134 subjects of 38 affected families and found no significant influence of foetal genotype. Estalella et al also described that while BW of patients with a GCK mutation was around the 10th centile of the population that of patients with a HNF-1α was similar to the mean of the population (111). Two publications in 2002 established that the parent of origin of the mutation influenced the age of presentation of DM in HNF-1α carriers. While Stride et al reported that age at diagnosis was 12 years earlier when the mutation was of maternal origin (115), Klupa et al described that in relation to carriers of mutations of paternal origin, those with a mutation of maternal origin displayed a shift to the left in the curves of cumulative risk of DM and the requirement for insulin (116).

TREATMENT

The fact that maternal origin of the mutation has an important impact on the age at presentation of DM in HNF-1α heterozygotes seems to put special emphasis on achieving near-normal maternal glycemic control during pregnancy. However, there is no information relating glucose tolerance in the offspring to maternal glucose control during pregnancy. Patients with a HNF-1α mutation show special sensitivity to sulfonylureas (117) because these drugs act on potassium channels of the beta cell, downstream of the defect in carbohydrate metabolism associated to the mutation. Taking this information, glyburide could be an option for treating women with a HNF-1α mutation presenting with GDM.

MUTATIONS IN THE HNF-4α GENE & GESTATIONAL DIABETES

The prevalence of mutations in HNF-4 α is presumed to be much lower than that of HNF-1α. Outside pregnancy, the following features have been identified as suggesting a HNF-4 α mutation and encouraging genetic testing for it in children and young adults with DM and a strong family history of DM (95). They share similar characteristics to HNF-1α but later onset and no early renal glycosuria and negative genetic testing for HNF-1α.

MACROSOMIA & NEONATAL HYPOGLYCEMIA

There is a single study addressing its prevalence in women with GDM with a positive family history of DM, the rate being 1%. However, in recent years important knowledge has been provided regarding the phenotype in the neonatal period of mutation carriers. The study by Pearson et al(114) clearly illustrates that newborns with the mutation displayed high BW and neonatal hyperinsulinemia. They studied 108 members of 15 families with the mutation, 54 of them affected. BW of infants with the mutation was on average 790 g higher, regardless of the origin of the mutation; the difference remained in

the 18 pairs of siblings discordant for the mutation. Regarding the rates of macrosomia, 13% of non-affected newborns had a BW over 4000 g vs 56% of affected newborns (64% when the mutation was inherited from the mother and 46% when inherited from the father). Six cases of large macrosomia (BW over 5000 g) were observed, all of them in affected newborns. Thus, in relation to GCK, the influence of HNF-4 α on BW is of opposite direction and more pronounced. In this study, neonatal hypoglycemia was reported in 8/54 mutation carriers (hyperinsulinemia documented in three, independent of the origin of the mutation) but in none of the 54 non-mutation carriers. In six subjects, neonatal hypoglycemia was transient but two patients required treatment with diazoxide and chlorothiazide for 1 and 6 months, respectively. These findings led the authors to hypothesize that a single genetic defect affecting the beta cell would produce hyperfunction in the foetal and neonatal period and defective beta cell function in adulthood. Additional reports have been published of HNF-4 α heterozygotes presenting neonatal macrosomia and neonatal hyperinsulinemic hypoglycemia either transient or persistent (118,119). This has led to the recommendation to test for HNF-4 α mutations in macrosomicnewborns with neonatal hypoglycemia that have a family history of youth-onset DM. However, it has recently been reported that this phenotype can also correspond to *de novo* HNF-4 α mutations (120).

OTHER RARE forms of MODY & GDM:

Genetic variations in the PDX1 (pancreatic-duodenal homeobox-1), MODY4 genealso known as insulin promoter factor-1 (IPF1) (MODY4) is not common but may contribute to early- or late-onset diabetes. Pdx-1, a homeodomain transcription factor, serves as a master regulator in the pancreas due to its importance during organogenesis and in adult islet insulin-producing beta cell activity. In an Italian study 40 early-onset type 2 diabetic probands were screened for IPF1 mutations, one of the extended family (viz., Italy-6) of 46 members with clinical phenotypes of gestational diabetes, MODY, and

T2D, a single nucleotide change of CCT to ACT was identified at codon 33 resulting in a Pro to Thr substitution (P33T) in the IPF1 transactivation domain. Gragnoli C et al in their study found that 4 out of the 5 female pregnant women who were carriers of PDX1 mutation, had their pregnancies complicated by reduced birth weights, miscarriages, or early postnatal deaths(121). These findings indicate that the P33T IPF1 mutation may be associated with the occurrence of gestational diabetes and MODY4. The IPF1 mutant protein (P33T) showed a reduction in DNA-binding and transcriptional activation functions as compared to the wild-type IPF1 protein in studies in vitro (121).

NEUROD1 mutations (MODY 6) have been reported from only six families(122–125). Although the direct pathogenic association with the clinical phenotype of some NEUROD1 variants remains unclear, the NEUROD1 mutation His241Gln has been reported in families with obesity(125). In a Norwegian study (94) on MODY X patients no NEUROD1 mutations were found and hence suggesting that NEUROD1 may not be a candidate gene in gestational diabetes mellitus (GDM) in that particular population.

Insulin gene (INS) mutations (MODY 10) are a common cause of permanent neonatal diabetes (PNDM) and a rare cause of diabetes diagnosed in childhood or adulthood. In a study of maturity-onset diabetes of the young (MODYX) patients and gestational diabetes mellitus (GDM) one novel heterozygous mutation c.17G>A, R6H, was identified in the pre-proinsulin gene (INS) in a Danish MODYX family(126) with one 27 year old GDM. They had around 30% reduction in beta-cell function (calculate by insulinogenic index). In a Czech MODYX family a previously described R46Q mutation was found and they were treated with oral hypoglycemic agents and/or insulin (126).

MODY8 can be caused by frameshift deletions in the variable number of tandem repeats (VNTR) of the carboxyl-ester lipase gene (CEL). The pancreas serves both endocrine and exocrine functions. The localization of the islets within exocrine pancreatic tissue is suggestive of an interdependency and crosstalk between these 2

cell populations in their normal and in their abnormal function. Raeder et al. (2006) described with several family members { with a frameshift in VNTR in the CEL gene) in 2 Norwegian kindreds(127) with and without diabetes who had mild abdominal pain and loose stools. Reduced pancreatic volume by computerized tomography of the pancreas was documented in all 10 mutation carriers with diabetes and exocrine deficiency(127).

KLF11 (MODY 7) interacts with the coactivator p300 via its zinc finger domain in vivo to mediate Pdx-1 activation, suggesting that gene regulation in MODY is more complex than anticipated previously(128). A common glutamine-to-arginine change at codon 62 (Q62R) in its gene *KLF11* has been recently associated with type 2 diabetes in two independent samples(OR = 1.29, P = 0.00033) but not in a separate European-derived population (OR 0.97 [95% CI 0.88–1.08], *P* = 0.63) (129).

MODY IN INDIA

Data about MODY in India is basic and preliminary. . In addition, all studies from India have assessed clinical prevalence or mutations in one MODY gene. The table 5 summarises all reported studies.

Table-5:The table below summarizes the studies in our country so far.

Investigators / Journal / Year / Reference	Mutations studied	Aims / Findings	Limitations
Mohan et al / *Diabetes Care* / 1985 / (130)	None	Estimated clinical prevalence based on clinical criteria by Tatersall&Fajans. 27% had autosomal dominant inheritance, while in 73% the mode of inheritance was not defined. Microvascular complications were common, and macrovascular complications were rare.	Genetic subtypes were not identified.
Anuradha S et al / *Diabetes Care* / 2005 / (16)	HNF1α	Estimated the prevalence of Ala98Val polymorphism in the HNF1α gene. It was significantly higher in MODY patients (p=0.0013), compared with control group.	Only one polymorphism in HNF1α was studied. There could be several polymorphisms in each gene, and several genes are implicated in MODY

Investigators / Journal / Year / Reference	Mutations		Investigators / Journal / Year / Reference
Radha V et al / *J. ClinEndocrinol Metabolism* / 2009 / (131)	HNF1α	Screening coding and promoter regions of HNF1α gene for mutations. They identified nine novel variants comprising seven mutations (one novel mutation -538>C at a promoter region and six novel coding region mutations) and two polymorphisms in HNF1α gene. Mutations in HNF1α gene comprised about 9% of clinically diagnosed MODY subjects.	They had looked at mutations in only one gene, whereas, several genes are implicated in MODY
Anuradha S et al / *Clin Genet* / 2011 / (132)	HNF4α	Screening coding and promoter regions of HNF4α mutations. Identified three novel mutations in the P2 promoter region of the gene, namely -1009G/C, -129T/C, and -79C/T. 3.4% of clinically diagnosed MODY subjects have MODY1.	Again, they studied only 1 gene, whereas several are involved in MODY
Sahu et al / *J Clin Endocrinol Metab* / 2007 / (17)	HNF1α, mitochondrial gene	Investigated the role of mutations in HNF1α, mitochondrial A3243G mutation, and islet autoimmunity in early onset Type2 DM. HNF1α mutation was detected in only 1 patient, mitochondrial A3243G mutation was present in one subject, and GAD antibodies were present in 3 subjects. The conclusion was that HNF1α mutations, mitochondrial mutations, and autoimmunity were infrequent in their population	This study was not uniquely focused on MODY, and does not contribute towards the other subtypes implicated in MODY

THE NECESSITY OF MOLECULAR DIAGNOSIS(133)

[1] CHARACTERIZING THE FREQUENCY OF THE DISEASE

Shields et al estimated that around 80% of the cases of MODY in UK are misdiagnosed as either Type 1 or Type 2 DM (134). While considering the high prevalence of Diabetes in India, these figures are more likely to be higher. There is a paucity of genetic diagnostic facilities in a developing country like India, due to which there has been a delay in deconstructing the stereotypic “Type 1 – Type 2” classification of diabetes. Therefore Indian clinicians and scientists are not equipped to deal adequately with the alarming epidemic of diabetes. It is imperative that we work towards establishing an affordable molecular genetic diagnostic platform for MODY, which would be a preliminary step in handling this global epidemic.

At our centre, a novel multiplex polymerase chain reaction (PCR) based target enrichment was established, followed by NGS on the Ion Torrent Personal Genome Machine (PGM) to identify mutations in a comprehensive panel of ten MODY genes. In a separate study, we included 80 subjects of Asian-Indian origin with young onset diabetes of whom 56 patients were clinically diagnosed as MODY. Mutations were identified in 21% (12/56) of the clinically diagnosed MODY subjects with a higher frequency of NeuroD1 mutations, a pattern distinct from the Western population. There were four mutations in NEUROD1 (novel c.175 G>C p.E59Q, novel c.-162G>A 5'UTR, and c.723C>G p.H241Q), two in HNF4A, two in PDX1, one each in HNF1A (c.505G>A p.V169I coseggregating with c.493-4G>A and c.493-20C>T), GCK (c.1318G>T p.E440X), HNF1B (novel c.274 C>T p.L92F) and PAX4 genes. Co-segregating NEUROD1-PDX1 mutations were detected in two patients (Figure-7). Novel Multiplex PCR coupled with Ion Torrent Next Generation sequencing has allowed us for the first time to perform comprehensive genetic screening in Asian Indians in a rapid and cost-effective way.

Figure-6:Details of MODY positive subjects

Subject Id	Sex	Age (yrs)	Age at diagnosis	BMI (kg/m2)	Clinical characters	Treatment	Gene	MODY	Mutation	Amino acid change	GERP	Comments
M010	M	23	21	25	3 generations affected	Metformin	HNF4A	MODY1	c.505G>A & c.493-4G>A c.493-20C>T	p.V169I		Reported mutation
M015	F	8	8	15	3 generations affected SGA, polyuria, vag candidiasis	Dietary modification	GCK	MODY2	c.1318G>T	p.E440X		Reported mutation
M001	F	21	11	19.6	3 generations affected	Insulin Glibenclamide	HNF1A	MODY3	c.1501G>T	p.A501S	4.49	Novel mutation
M060	M	34	28	36.4	3 generations affected Obese	Metformin Gliclazide	PDX1	MODY4	c.302C>T	p.P101L	2.15	Novel mutation
M062	M	40	26	24.4	3 generations affected DKA+, Neuropathy, retinopathy and nephropathy	Insulin	PDX1	MODY4	c.529G>A	p.V177M	4.86	Novel mutation
M019	F	27	25	26.3	3 generations affected	Metformin Insulin (pregnancy)	HNF1β	MODY5	c.274C>T	p.L92F	5.13	Novel mutation
M018	M	30	30	19.3	2 generations affected	Glimepiride	NEUROD1	MODY6	c.175 G>C	p.E59Q	5.9	Novel mutation
M026	M	47	28	22.8	2 generations affected Hypertension, single vessel CAD, Mild Non proliferative diabetic retinopathy and nephropathy	Glimepiride	NEUROD1	MODY6	c.723C>G	p.H241Q	4.19	Reported mutation - Co-segregated with PDX1 rare variant
							PDX1		g.G>A rs137852787	p.E224K	4.01	Clinically pathogenic rare variant
M030	M	30	30	27.4	3 generations affected	Metformin Glipizide	NEUROD1	MODY6	c.-162G>A 5'UTR		4.05	Novel mutation Co-segregated with PDX1 rare variant
							PDX1		g.G>A rs137852787	p.E224K	4.01	Clinically pathogenic rare variant
M047	F	35	24	39.7	3 generations affected	Metformin Glimepiride	NEUROD1	MODY6	c.723C>G	p.H241Q	4.19	Co-segregated with obesity

[This was presented at the 63rd Annual Meeting of the American Society of Human Genetics held on October 22-26, 2013, Boston, MA. www.ashg.org/2013meeting/abstracts/fulltext/f130122650.htm]

THE IMPACT OF CONSANGUINUTY AND THE FREQUENCY OF MODY

Consanguinity is also an issue which will have a bearing on our study. It is a common practice in Tamil Nadu. In 2001, data from the 1992-1993 National Family Health Survey was used to assess trends in consanguinity in South Indian states (70). In Tamil Nadu, the preferred Dravidian system was uncle-niece marriage. In Kerala, this was conspicuously absent. It was observed that in Karnataka and Andhra Pradesh, as well, that the consanguinity and co-efficient of breeding was high. Given the genetic mechanism implicated in diabetes, our study might reveal a potentially increased frequency of MODY gene mutations in our study population.

[2] ESTABLISHING A DIRECTION FOR APPLIED PHARMACOGENOMICS

Patients with mutations in HNF4α (MODY1) and HNF1α (MODY3) respond well to sulphonylureas alone, while mutations in GCK (MODY2) are managed with diet alone, and rarely require pharmacotherapy. Mutations in the HNF1β gene results in severe beta-cell dysfunction and these patients require insulin right from a very early stage of the disease. Even though one can diagnostically suspect MODY based on the phenotypical features we have mentioned earlier, one cannot treat the patient without knowing the specific genotype. The specific management protocols for each genetic subtype mandates genetic analysis in patients with clinical suspicion of MODY.

[3] AVOIDING INSULIN THERAPY

Given the fact that MODY is misdiagnosed as either Type 1 or Type 2, the patients end up receiving Insulin, along with the complications of insulin therapy, and manage to achieve only suboptimal glycemic control (135). This can be avoided, and treatment can be streamlined if genetic subtype is known through molecular genetic analysis and the family is screened for the same mutation. The response to therapy in the family members will guide in the therapy of such patients.

Although most cases of gestational diabetes resolve with delivery, the definition applied whether or not the condition persisted after pregnancy and did not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with pregnancy. GDM is considered to result when the genetic predisposition is triggered by increased insulin resistance during pregnancy leading to what seems to be one of the primary characteristics of GDM, the pancreatic beta-cell impairment. Old age, obesity and a high fat diet represent some important non-genetic factors contributing to insulin resistance(136). There is now increasing evidence from animal and epidemiologic studies that prenatal exposure to a hyperglycemic environment can alter the growth trajectories and homeostatic regulatory mechanisms, causing lifelong changes resulting in increased risks for obesity and metabolic and cardiovascular diseases in the offspring (137,138).

For a given population and ethnicity, the prevalence of GDM corresponds to the prevalence of impaired glucose tolerance [IGT], in a non-pregnant adult within that given population (5). There has also been a trend towards a shift in the age of onset of diabetes (perceived as T2D) to a much younger age, that ranges between 25 to 34 years (11). In late pregnancy, insulin sensitivity falls by ~50%, contributed by increased maternal adiposity and the insulin desensitizing effects of hormones produced by the placenta. Relatively high glucose levels during and soon after pregnancy also correlate with increased risk of diabetes, perhaps because they identify women who are relatively close to developing diabetes when the diagnosis of GDM is made. Hence, it has been suggested that women who develop GDM early (< 20 weeks) may also have an underlying deficit in this additional insulin secretory [beta cell dysfunction]. Beta-cell dysfunction in these women may fall into one of three major categories: *1*) occurring on a background of pre-existing insulin resistance (as is most common), *2*) autoimmune or *3*) monogenic. Lean patients are more likely have autoimmune or monogenic forms of

diabetes. The MODY genes may contribute to turning on an early onset of diabetes in pregnant women with GDM and overt diabetes and account for < 10% of GDM cases(56). The current approach to the molecular diagnosis of MODY includes the screening of few MODY related genetic mutations (hepatocyte nuclear factor 1alpha (HNF1A), hepatocyte nuclear factor 4 alpha (HNF4A) and Glucokinase (GCK)) in a sequential pattern(139). In a developing country like India, the lack of awareness and limited facilities for genetic diagnosis may result in patients receiving inappropriate therapy with suboptimal glycemic control. With the advent of next-generation sequencing (NGS) technology, there has been a dramatic improvement in cost, speed and scalability of sequencing(20,21). Hence based on our data from the MODY project done at our centre showing a wide spectrum of causative MODY mutations in contrast to earlier reports we screened *pregnant women with diabetes* for a comprehensive panel of 10 MODY genes consisting of HNF1A, HNF4A, GCK, HNF1β, IPF1, NEUROD1, KLF11, CEL, PAX4, and INS. We believe that this will have a significant impact on both maternal and foetal health.

GESTATIONAL DIABETESAND SUSCEPTIBILITY GENE TCF7L2

TRANSCRIPTION FACTOR 7-LIKE 2 (TCF7L2)

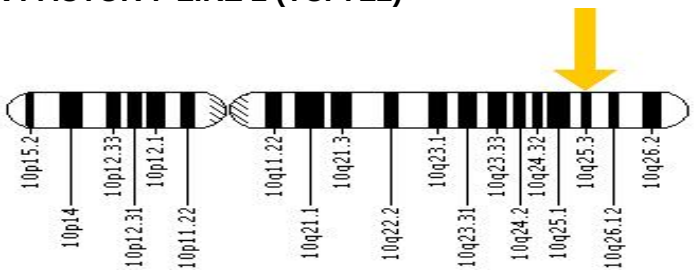


Figure 7: shows location of TCF7L2 in chromosome 10

The TCF7L2 gene is located on the long (q) arm of Chromosome 10 at position 25.3.More precisely, the TCF7L2 gene is located from base pair 114,710,008 to base pair 114,927,436 on chromosome 10.The transcription factor 7-like 2 (TCF7L2) is a member of the Wntsignaling pathway, but its role in the pathogenesis of diabetes is not well understood.

Recent studies have shown a consistent and strong association between variants of TCF7L2 and T2D potentially through a mechanism involving insulin secretion. The association between TCF7L2 variants and GDM has been studied in different populations. TCF7L2 has showed modest evidence for association with GDM, with P-values ranging between 0.038 and 0.003. Similar to the study of Cho et al. [52], Lauenborg and colleagues [51] examined the association between 11 T2DM susceptibility loci for association with GDM in a Danish case-control sample. In their study [51], only TCF7L2 showed strong evidence for association with GDM, with an odds ratio similar to that observed for T2DM. Shaat et al. found that the T allele of the rs7903146 (IVS3C_T) variant was associated with an increased risk for GDM among 1881 unrelated pregnant Scandinavian women (649 with GDM and 1232 nondiabetic controls).Watanabe et al. observed that the T allele of the rs12255372 variant was more frequent among 94 Mexican-American women with previous GDM compared with 58 control subjects. These results show that TCF7L2 may be involved in GDM.

TCF7L2 although they lie in the intron, these genetic regions might be important for gene regulation. In TCF7L2, there is an alternative splicing exon near to the SNP hence this might be related. Generally, non-coding SNPs manipulate gene expression because; these SNPs appear in gene regulatory sequences, like promoters, enhancers and insulators. If TCF7L2 gene expression is regulated by non-coding SNP, then the sequence where the SNP appears to be important region and needs to be studied(9). TCF7L2, the susceptibility gene with the largest effect on disease susceptibility discovered to date, was identified pre–genome-wide association by Grant et al. in 2006, with rapid replication of its consequence on diabetes susceptibility in multiple populations. TCF7L2 was a positional candidate gene that mapped to a region of genetic linkage to type 2 diabetes in the Icelandic population on chromosome 10.

Chandak et al has genotyped type 2 diabetes patients (n=955) and ethnically matched control subjects (n=399) by sequencing three single nucleotide polymorphisms (SNPs) (rs7903146, rs12255372 and rs4506565) in TCF7L2(31). And he has observed a strong association with all the polymorphisms, including rs12255372 (odds ratio [OR] 1.50 [95% CI=1.24–1.82], p=4.0×10−5), rs4506565 (OR 1.48 [95% CI=1.24–1.77], p=2.0×10−5) and rs7903146 (OR 1.46 [95% CI=1.22–1.75], p=3.0×10−5). All three variants showed increased relative risk when homozygous rather than heterozygous, with the strongest risk for rs12255372 (OR 2.28 [95% CI=1.40–3.72] vs OR 1.43 [95% CI=1.11–1.83]). We investigated to find whether these TCF7L2 variants (rs7903146, rs12255372 and rs4506565) are also associated with Gestational diabetes mellitus in the South Indian population.

Table-6: The table below summarizes the data from India.

Investigators / Journal / Year / Reference	Aims	Polymorphisms studied	Findings
Chandak et al / *Diabetologia* / 2007, 50: 63-67 (141)	Investigating whether TCF7L2 variants are associated with diabetes in the Indian population.	rs7903146, rs12255372, rs4506565	All three variants showed increased relative risk with homozygous rather than heterozygous with strongest risk for rs12255372
Bodhini et al / *Metabolism* / 2007 Sep; 56(9):1174-8 / (142)	Association of rs12255372(G/T) and rs7903146(C/T) polymorphisms of the (TCF7L2) gene with type 2 diabetes mellitus in Asian Indians.	rs12255372(G/T) rs7903146(C/T)	The frequency of the "T" allele of both rs12255372 (G/T) and rs7903146(C/T) polymorphisms was significantly higher in diabetic subjects (23% and 33%) compared to that in normal glucose-tolerant subjects
Chauhan et al / *Diabetes* / Vol 59, August 2010, 2068-2074 / (143)	To check the association of common variants in 8 susceptibility genes (PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2A, IGF2BP2, CDKAL1) with Type 2 Diabetes	PPARG- rs1801282 KCNJ11 -rs5219 TCF7L2 -rs7903146 SLC30A8-rs13266634 HHEX -rs1111875 CDKN2A-rs10811661 IGF2BP2-rs4402960 CDKAL1 -rs10946398	Confirmed association of all 8 loci with type 2 diabetes with odds ration ranging from 1.18 to 1.89 ($P=1.6 \times 10^{-3}$ to 4.6×10^{-34}). The strongest association and highest effect size was observed for TCF7L2.
Janipalli et al / *Diabetic Medicine* / 2012 Jan / 29(1):121-7 / (144)	Investigated the effect of susceptibility genetic variants in Type 2 Diabetic patients	Studied 32 variants in 19 susceptibility genes	Allelic odds ratio varied from 1.01 (95% CI 0.85 to 1.19) to 1.66 (95% CI 1.32 to 2.01).

The above studies have looked at the influence of TCF7L2 in Type 2 diabetes, and have demonstrated a strong association between the two. However, In India till date there is no study which looked at the role of genetic variants of TCF7L2 in GDM.As we know that GDM and T2D share a common pathophysiological features, we would like to investigate the association between GDM and common T2D susceptibility polymorphism of TCF7L2. Our study would reveal the magnitude of influence of genetic variants in TCF7L2 on augmenting the clinical development of gestational diabetes mellitus.

Study design: Cross-sectional observational study

This study was approved by the Institutional Review Board (IRB) and Ethics Committee of Christian Medical College, Vellore. (IRB.Min.No:7998 dated 08.09.2012). Written informed consent was obtained from all healthy control subjects and study participants.

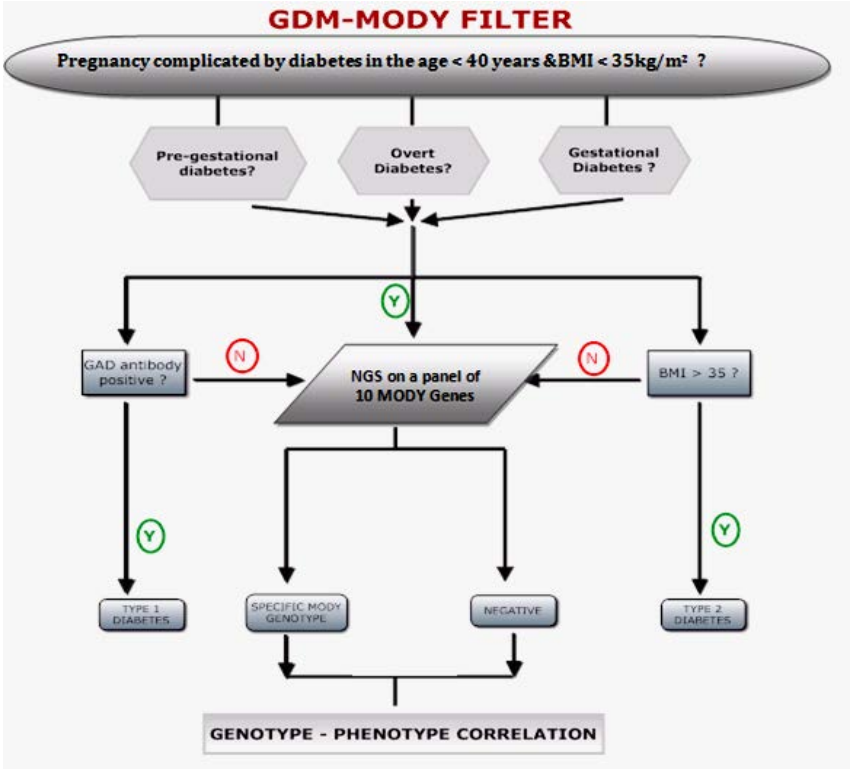
SECTION-I:

Study Population

Between September 2012 to December 2013, we selected a total of 50 subjects [women with pregnancy complicated by hyperglycemic disorders] for genetic analysis. Subjects who fulfil the inclusion criteria were assessed through a detailed questionnaire including an extensive pedigree (at least 3 generations) and physical examination. The questionnaire included information on age at diagnosis, symptoms at onset, ketoacidosis at onset or later, glycemic profile, treatment received with exact duration and other comorbidities. The initial glycemic status was assessed by HbA1c levels, either fasting, 2 hour post meal plasma glucose or 75 gram oral glucose tolerance test in pregnant women with hyperglycemic disorders. GAD (glutamic acid decarboxylase) antibodies were done to exclude typical type 1 diabetes from antibody negative forms.

Anthropometric measurements included height, weight and BMI. The body mass index (BMI) was calculated using the formula Bmi=weight (in kilograms)/ height (in m^2) based on the pre-conceptional weight or weight recorded at the initial visit in first trimester. All subjects underwent at least one check-up by an Ophthalmologist during the study period. Plasma glucose (fasting) is measured by enzymatic method (hexokinase). The biochemical assays are done on a Hitachi 912 auto analyser using reagents from Roche Diagnostics (Mannheim, Germany) with the appropriate quality control methods. GAD antibodies are measured with radioimmunoassay technique.

The outcomes involved are studied at the clinical, biochemical, and genetic levels. Clinically, the phenotypical characteristics were correlated with the specific genotypes. This includes the age of onset, family history, BMI, outcome of pregnancy, birth weight of baby,cesarean delivery, clinical neonatal hypoglycemia, premature delivery (before 37 weeks of gestation). Genetically, the outcome involved the different mutations that are implicated that were detected after sequencing the genes. Potential confounders and effect modifiers are those patients who cannot be detected by the GDM-MODY FILTER, and those who have a false diagnosis of MODY as well. To begin with, MODY has an ambiguous clinical presentation as we have explained above. Thus, there is a possibility that we can miss patients (who actually have MODY) on both sides of the spectrum (that is, either on the side of Type 1 or Type 2 diabetes), and also end up with patients (who do not actually have MODY), because of their overlapping clinical presentation. **GDM - MODY FILTER -** The flowchart in the next page depicts the method used for screening patients into our study.



Inclusion Criteria

- All pregnant women with any degree glucose intolerance and diabetes at any stage of pregnancy
- Age of onset of disease <40 years

We have altered the age to 40 years to study the MODY genetic mutations, as opposed to 25 years suggested by Tattersall due to phenotypic heterogeneity (14),(79),(134) and their diabetes may also remain undiagnosed due to lack of screening and testing though they may have had the disease from a much earlier age. We also believe many of them may not have symptoms and that the stress of pregnancy may uncover the pre-existing glucose intolerance due to genetic mutations that result in beta-cell dysfunction.

Exclusion Criteria

- Type 1 DM patients and those who are GAD antibody positive.
- Type 2 DM patients, who have age of onset of diabetes >40 years and/or are obese with BMI > 35 kg/m^2
- Any subject with secondary diabetes, glucocorticoid use, lipodystrophy, fibrocalcific pancreatic diabetes
- Patients who refuse to give their consent or blood for sampling

CLASSIFICATION OF HYPERGLYCEMIC DISORDERS COMPLICATING PREGNANCY: (Diabetes Care March 2010 vol. 33 no. 3 676-682)

1. **GESTATIONAL DIABETES MELLITUS [GDM] :**

   Plasma glucose values lower than those diagnostic of diabetes.

2. **OVERT DIABETES MELLITUS IN PREGNANCY [OVERT]:**

   Glucose values / HbA1c diagnostic of diabetes using the standard American Diabetes Association (ADA) Criteria at the first visitany time after conception.

3. **PRE-GESTATIONAL DIABETES MELLITUS [PREGDM] :**

   Pregnant women who were diagnosed to have diabetes prior to conception.

Gestational Diabetes Mellitus (GDM):

The diagnosis of GDM is made when any of the following plasma glucose values are exceeded after a 75-g oral glucose tolerance test (OGTT) any time during pregnancy.

a) Fasting: ≥92 mg/dl (5.1 mmol/l)
b) 1 h: ≥180 mg/dl (10.0 mmol/l)
c) 2 h: ≥153 mg/dl (8.5 mmol/l)

[**NOTE**: All women not previously diagnosed with overt diabetes underwent a 75-g oral glucose tolerance test (OGTT) at 24-28 weeks of gestation. The OGTT was performed in the morning after an overnight fast of at least 8 h. As per the IADPSG(145) Consensus Panel OGTT was not routinely performed before 24–28 weeks' gestation but a fasting plasma glucose value (lower than those diagnostic of diabetes) in early pregnancy (92 mg/dl) was also classified as GDM.]

Overt Diabetes Mellitus in pregnancy (OVERT): For many years, GDM has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy. After deliberations in 2008–2009, the International Association of Diabetes and Pregnancy Study Groups (IADPSG), an international consensus group with representatives from multiple obstetrical and diabetes organizations, including the American Diabetes Association (ADA), in 2010 recommended that high-risk women found to have hyperglycemia in the diabetes range at their initial prenatal visit, using the standard criteria receive a diagnosis of "*Overt*", not gestational diabetes(145) and this has been followed in our study

Standard criteria by American Diabetes Association (ADA) for the diagnosis of diabetes(57)**:**

AIC $\geq$ 6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.

OR

FPG $\geq$ 126mg%. Fasting is defined as no caloric intake for at least 8 hours. *

OR

2-h plasma glucose > 200mg% during an OGTT. The test should be performed as described the World Health Organization, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.*

OR

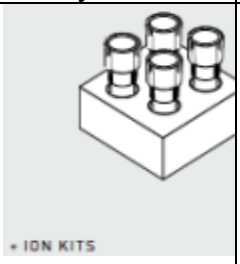
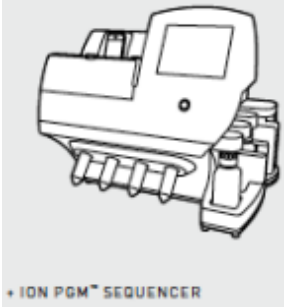
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose $\geq$ 200mg%.

*In the absence of unequivocal hyperglycemia, criteria 1-3 should be confirmed by repeat testing.

GENE SEQUENCING:

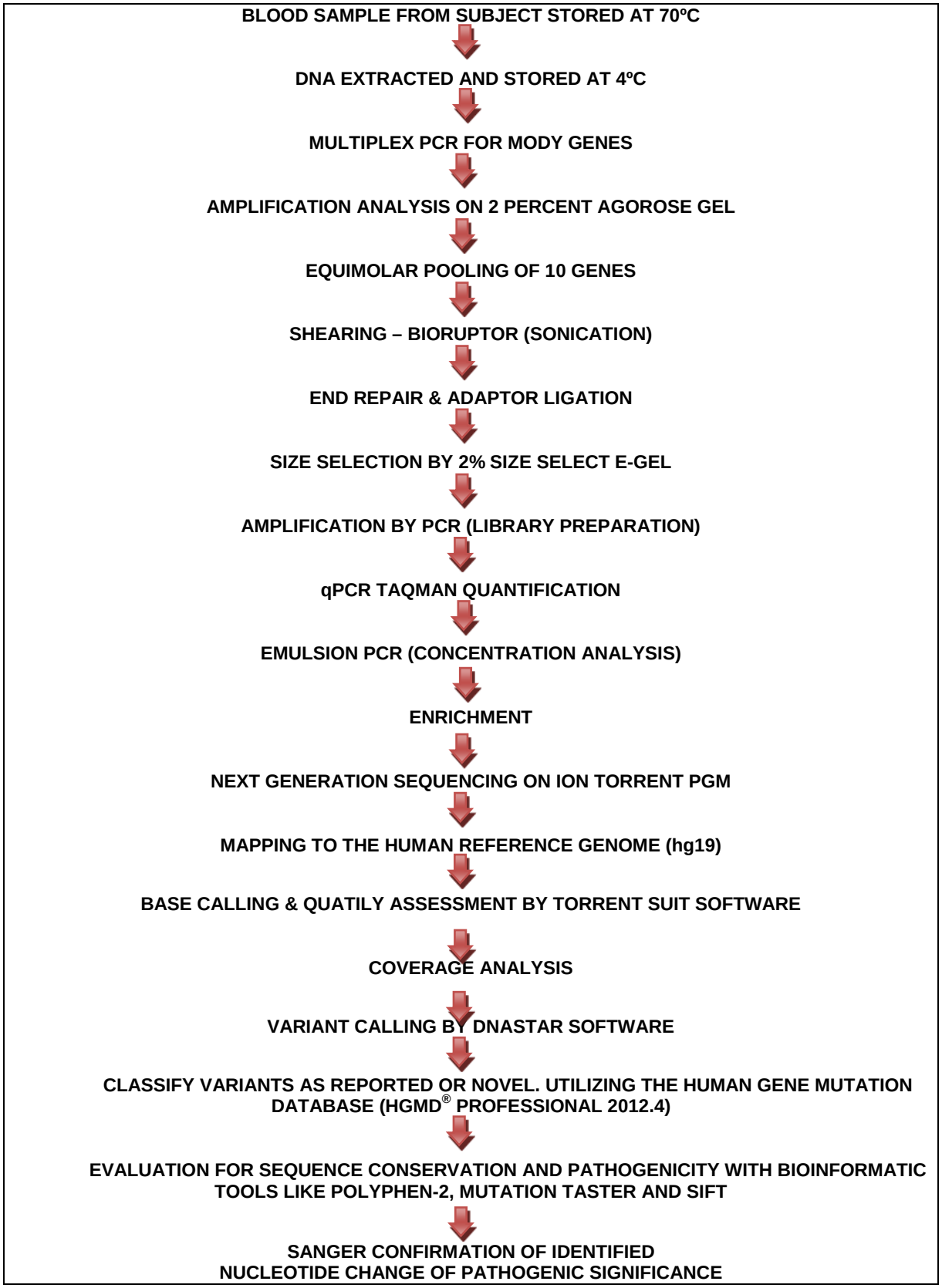
Sanger sequencing is currently the gold standard to diagnose the genetic variation and detect mutations within disease genes. Mutations in more than 10 different genes have been implicated in MODY. However, because of the genetic heterogeneity and limitations in throughput and scalability of current diagnostic tools, up-till now it has not been possible to genetically characterize MODY in patients with gestational diabetes in a fast, comprehensive and cost-effective manner. In this study we screened 50 pregnant women with diabetes, for mutations in a comprehensive panel of 10 MODY genes [HNF1A, HNF4A, GCK, HNF1β, IPF1, NEUROD1, KLF11, CEL, PAX4, and INS} using the next-generation sequencing platform to provide a fast and cost-efficient diagnosis of MODY.

SCHEMATIC REPRESENTATION OF NGS WORKFLOW

STEPS	**1**	**2**	**3**	**4**
PROCESS	CONSTRUCT LIBRARY	PREPARE TEMPLATE	RUN SEQUENCE	ANALYZE DATA
DETAILS	Genes of interest are amplified by novel Multiplex PCRs, and fragmentation is done by Ion Express Plus Library Kit.	Template prepared using Emulsion PCR, on Ion One Touch System	The sequences are run using the Ion PGM Sequencer, which uses the Ion 314 or 316 chip	Data is analyzed using Torrent Suite 1.5 software
EQUIPMENT USED AT VARIOUS STAGES				

(Pictures by Life Technologies, California, USA. Taken from: http://www.invitrogen.com/site/us/en/home/Products-and Services/Applications/Sequencing/Semiconductor-Sequencing/pgm.html)

FLOW CHART FOR VARIANT ANALYSIS BYNEXT GENERATION SEQUENCING



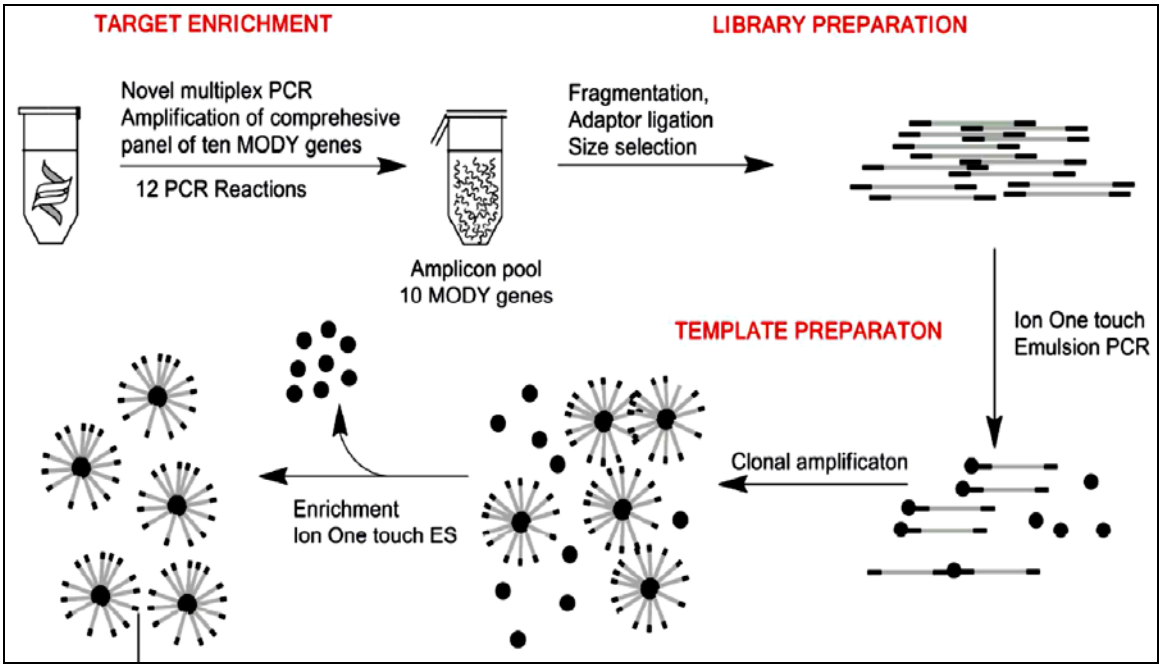
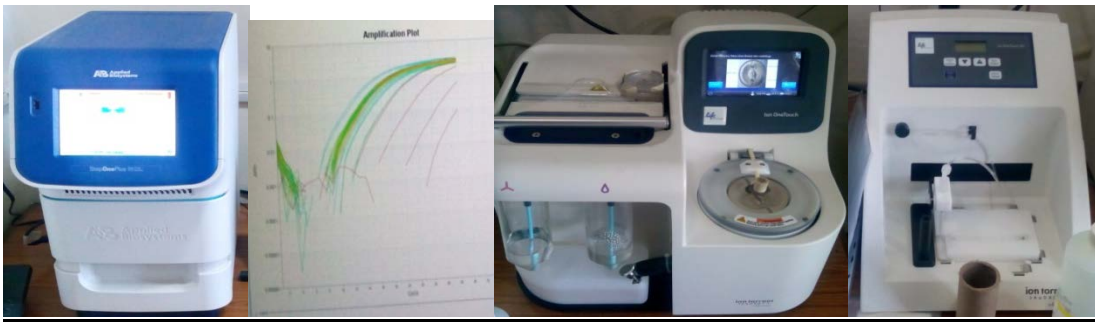


Figure-8: 2nd Generation Genetic Diagnosis of MODY work flow. 1. Target Enrichment: Novel multiplex PCR approach to amplify ten genes in 12 PCR reactions.2. Library Preparation: Ion Xpress™ Plus Fragment Library Kit was utilized to shear the amplicon pool, adaptor ligation, size selection with 2% egel size select followed by amplification. Agilent 2100 DNA High sensitivity chip and TaqMan assay was utilized for quantification of the libraries. 3. Template preparation: Template preparation was performed on Ion One Touch Emulsion PCR system, followed by enrichment on Ion One Touch Enrichment System. (Courtesy Aaron Chapla, Senior Research officer, Molecular Lab, Endocrinology, CMC, Vellore).

– 70^0 C ULTRA FREEZER, AMPURE BEAD CLEANUP OF DNA, REAL TIME TRANS-ILLUMINATOR& BIORUPTOR



REAL TIME PCR, AMPLIFICATION PLOT FOR LIBRARY, EMULSION PCR, ENRICHMENT SYSTEM [ION ONETOUCH]

MATERIALS & METHODS

We chose the Ion Personal Genome Machine (PGM) system, as this is one of the more proficient Next-Generation Sequencing platforms, which provide the very rapid sequencing run times with flexible and scalable solutions to fit our dynamic research needs. With simple workflows and scalable solutions, Ion semiconductor sequencing brings a variety of options to complete targeted re-sequencing projects accurately, quickly, and affordably. Furthermore the multiple options for library preparation allows one to study amplicons of various lengths, Ion Bar-coding kits enable sample multiplexing, and multiple Ion sequencing chips support sequencing target regions of variable sizes.

ION TORRENT PERSONAL GENOME MACHINE (PGM)

314 ION CHIP

4 NUCLEOTIDE KITS FOR NGS

Next generation sequencing: Performed on Ion Torrent PGM with 314 Ion chips and 200bp sequencing kits. Ion torrent suit software and DNA star software was utilized for data analysis. Sanger sequencing was performed to validate the protocol and the results.

DNA Sequencing on NGS platform:

Step: 1. *Target enrichment using Multiplex PCR*

The specificity and uniformity of the polymerase chain reaction (PCR) based enrichment strategy would always exceed that of hybrid capture(146). Therefore, we developed a novel multiplex PCR based enrichment for ten MODY genes using a QIAGEN® Multiplex PCR kit, followed by sequencing on an Ion Torrent™ PGM. The panel of genes included HNF1α, HNF4A, GCK, hepatocyte Nuclear factor 1beta (HNF1B), insulin promoter factor 1 (IPF1), neurogenic differentiation factor 1 (NEUROD1), kruppel-like factor 11 (KLF11), carboxyl ester lipase (CEL), paired box 4 (PAX4) and insulin (INS). The Primer 3 software was used to design 57 primer sets covering the 34.4kb target of these ten genes (except the pseudo gene regions of the CEL gene). The primers included an additional 50bp upstream and downstream of each exon to capture the splice junctions and also the 5´ UTR and 3´UTR regions. Furthermore, these primers were designed in such a way as to exclude the known polymorphic sites, thereby reducing allelic dropout rates.

Library preparation

Enzymatic shearing of pooled amplicons, adaptor ligation, nick repair and amplification was performed using the Ion Xpress™ Plus Fragment Library Kit (Ion Torrent, Life Technologies). The sheared DNA was further ligated with common adaptors for a single sample and Ion Xpress™ Barcode Adapters (Ion Torrent, Life Technologies) for the multiplex samples. The ligated samples were size selected on E-Gel® Size Select™ 2% pre-cast agarose gels. The Agilent bioanalyzer high sensitivity DNA chip (Agilent Technologies, Santa Clara, CA, USA) was used to determine the quality and concentration of the libraries, the concentration was further confirmed by a TaqMan® Quantitation Kit (Ion Torrent, Life Technologies).

Template preparation

The clonal amplification (template preparation) was carried out on an Ion OneTouch Emulsion PCR using the Ion OneTouch™ 200 Template Kit (Ion Torrent, Life Technologies). This was followed by a streptavidin-coupled Dynabeads® based enrichment on an Ion OneTouch ES (Ion Torrent, Life Technologies) prior to sequencing.

Sequencing using Ion 314 and 316 chips and Bioinformatic analysis

Sequencing was performed on an Ion torrent PGM using Ion PGM™ 200 Sequencing Kit (Ion Torrent, Life Technologies), utilizing 314 chips (multiplex 3 samples) and 316 chips (multiplex 8 to 10 samples). The generated sequencing data was mapped to the human genome reference hg19 using Torrent Mapping Alignment Program (TMAP). The Torrent suit software with v2.0 and/or v3.6.2 was used for all analysis. The coverage analysis was calculated using Torrent Coverage Analysis and potential pathogenic variants were identified using the Torrent Variant Caller. In addition, we utilized DNA Star software (DNASTAR, Madison, WI, USA) for further data analysis.

Validation:

Sanger's sequencing was done to confirm all the identified mutations and rare variants. In addition, to validate this protocol and Ion PGM sensitivity and specificity, we compared Sanger sequencing and Ion PGM data of the HNF1α gene in ten samples (M10 to M19) (Table 2 in the supplementary data). The NGS data was 100% concordant with Sanger sequencing data without any false positive single nucleotide variants calls. All the novel variants were evaluated for sequence conservation and likelihood of pathogenicity with PolyPhen-2(147), Sorting Intolerant From Tolerant (SIFT)(148), Mutation Taster(149) and Genomic Evolutionary Rate Profiling (GERP)(150). The Human Gene Mutation Database (HGMD® Professional 2012.4) was utilized to classify the identified variants as reported or novel.

Statistical analysis:

Deriving the sample size for screening of the ten MODY genes through standard methods was not possible due to the lack of prevalence data on outcome measures from the previous studies in the country. Limited data exists on the mutation rate of genes from similar population. Based on the literature review, the prevalence of MODY in relation to gestational diabetes was reported to range from 1% to 80% in the western population based on screening for the three common MODY genes (HNF4A, GCK and HNF1A). Therefore, as a preliminary study,considering the high prevalence of both diabetes {overall prevalence in India is around 6-8% and Tamil Nadu ~10.4%}(11)and gestational diabetes in India (overall 4-18% in India and 17.8% women in urban,13.8% in semi urban and 9.9% in rural areas in the state of Tamil Nadu(3)(4) and also study of a panel of 10 MODY genes instead of three, we presumed that the middle value as the prevalence of MODY i.e. nearly 40%, when all the 10 MODY genes were taken together. Assuming a mutation rate of 40%, with varying precisions and 95% confidence interval (CI) , the calculated sample size using the **N-mastersoftware** was as follows:

Single Proportion - Absolute Precision

Expected Proportion of all the 10 Genes MODY in GDM	40%	40%	40%
Precision (%)	10	12	15
Desired confidence level (1- alpha) %	95	95	95
REQUIRED SAMPLE SIZE	**92**	**64**	**41**

The above table suggest the numbers with the prevalence of 40% with 95% CI but with varying precisions. However, we studied 50 subjects that provide a precision between 12 to 15%.

SECTION II: Genotyping for TCF7L2 polymorphism in subjects with GDM.

During the period September-2012 to December- 2013, DNA extraction was done in 166 consecutive unrelated women (117 women with gestational diabetes mellitus, 49 non-diabetic control subjects) using the Gentrapuregene blood method. The primers were validated by Sanger sequencing (3130 genetic analyzer) and genotyped 3 TCF7L2 polymorphisms (rs7903146, rs12255372 and rs4506565) using a TaqMan allelic discrimination assay.

STEPS:

1. Extract DNA from the Whole Blood
2. Validate Primers before genotyping by Sanger sequencing
3. Perform SNP(single nucleotide polymorphism) Genotyping using Taqman Assays
4. Analyse which of these polymorphisms has a major effect in predisposition of the gestational diabetes

1. DNA Extraction:

1. Dispense 13 ml RBC Lysis Solution into a 15 ml centrifuge tube

2. Add 3 ml whole blood, and mix by inverting 10 times.

3. Incubate 5 min, at room temperature (15–25°C). Invert atleast once during the incubation.

4. Centrifuge for 10 min at 4000 rpm to pellet the white blood cells.

5. Carefully discard the supernatant by pipetting or pouring, leaving approximately 200 µl of the residual liquid and the white blood cell pellet.

6. Vortex the tube vigorously to resuspend the pellet in the residual liquid.

7. Add 2 ml Cell Lysis Solution, and pipet up and down to lyse the cells or vortex vigorously for 10 s.

9. Add 1 ml Protein Precipitation Solution, and vortex vigorously for 20 s at high speed.

10. Centrifuge for 7 min at 4000rpm

The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.

11. Pippete 4ml of alcohol into a clean 15 ml tube, and add the supernatant from the previous step by pouring carefully. Be sure the protein pellet is not dislodged during pouring.

12. Mix by inverting gently 50 times until the DNA is visible as threads or a clump.

14. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.

15. Add 1 ml of 70% ethanol and invert several times to wash the DNA pellet.

Carefully transfer to a new 1.5 ml tube

18. Add 200 µl DNA Hydration

19. Incubate at 37°C overnight and store it at -20^{0}c

PCR Reaction include the following

Master Mix-7.5 µl,Forward primer-0.5 µl,Reverse primer-0.5 µl,Nuclease free water-5.5 µl and DNA-1µl

The PCR conditions were standardized using the Takara Master Mix...

The PCR conditions starts with Initial denaturation 95^{0}C for 5 min followed by

98^{0}C-10 sec Denaturation
60^{0}C-30 sec Annealing } 30 cycles
72^{0}C-1 min Elongation

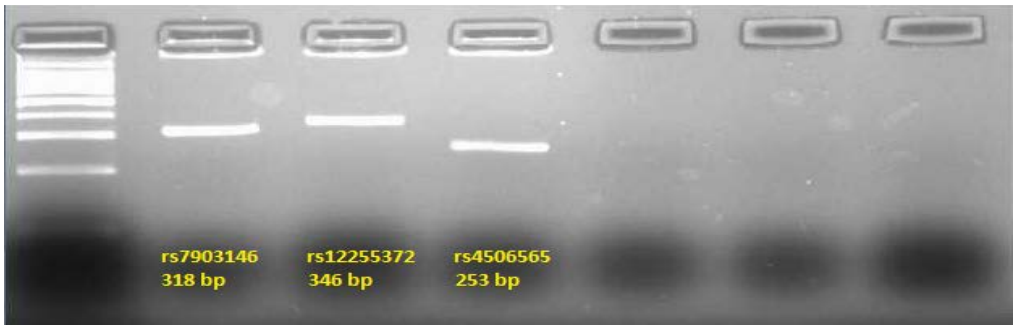
72^{0}C-5 min

Magnetic bead clean up:

1. 1 : 1.8 ratio of sample to AGENCOURT® AMPURE® XP beads were used .
2. So for 15µl reaction we have used 27 µl of magnetic beads.(mix it well)
3. After 5 mins incubation in room temperature. Remove the supernatant and Wash the Magnetic beads with 70% alcohol for 2 times.
4. Air dry it for few minutes and then elute it with 25 µl Nuclease free water

5. Run the samples in the gel and based on the intensity of the band dilute the sample do sequencing reaction

Figure-9: Amplification of the three polymorphisms on 2% Agarose Gel



PCR sequencing (Reaction setup)

Forward Primer-1.6 µl,Product(DNA sample)-2 µl,Buffer-1 µl,R.R. mix(Big dye ® Terminator v3.1 -0.5 µl and Nuclease free Water-4.9 µl

PCR program

96^0c-15 sec	Denaturation	25 cycles
50^0c-20 sec	Annealinjg	
60^0c -4 min	Elongation	

Post clean up in Millipore membrane filter:

2. Validation by Sanger Sequencing-Primer:

The Primers have been validated by Sanger Sequencing to confirm the presence heterozygous alleles :

Colour code: A-green, T-red, G-black , C-blue

Figure-10A: Variation (C/T) TCF7L2 Polymorphism rs7903146

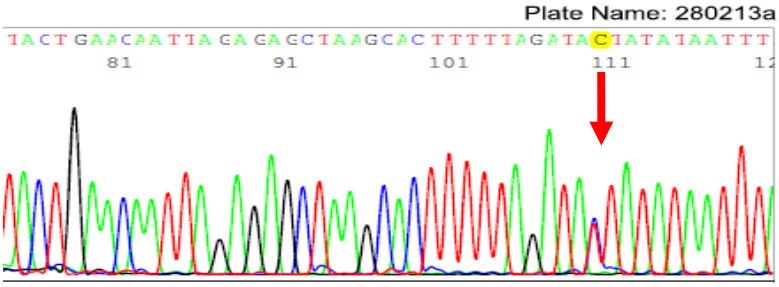


Figure-10B: Variation (G/T) TCF7L2 Polymorphism rs12255372

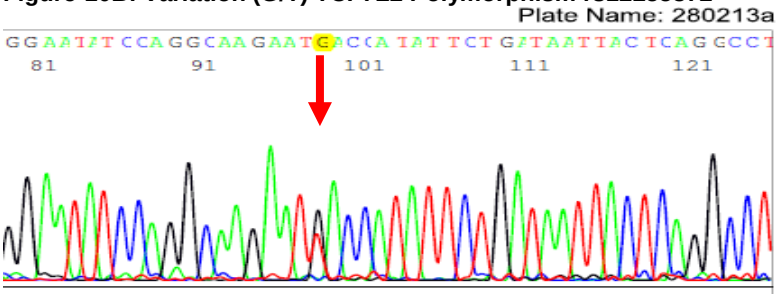
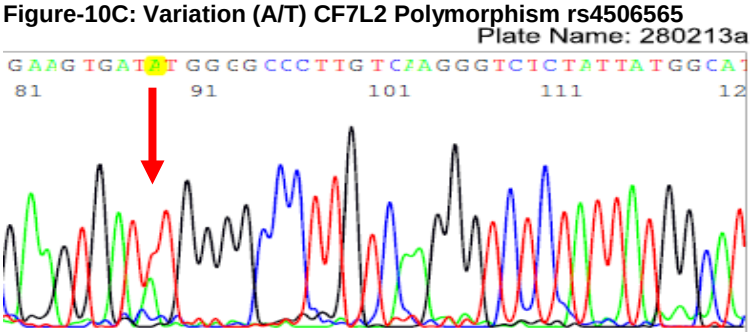


Figure-10C: Variation (A/T) CF7L2 Polymorphism rs4506565



3. Utilizing Taqman assay SNP Genotyping was done: Taqman-based SNP genotyping(TCF7L2 Variant Genotyping- Dennis T. Villareal et al)

The TaqMan® based SNP genotyping technology utilizes the 5' nuclease activity of *Taq* polymerase to generate a fluorescent signal during PCR. For each SNP, the assay uses two TaqMan® probes that differ in sequence only at the SNP site, with one probe complementary to the wild-type allele and the other to the variant allele. The technique utilizes the FRET technology whereby a 5' reporter dye and a 3' quencher dye are covalently linked to the wild-type and variant allele probes. When the probes are intact, fluorescence is suppressed because the quencher dyes are in the proximity of the reporter dyes. In the PCR annealing step, the TaqMan® probes hybridize to the targeted SNP site. During PCR extension, the reporter and quencher dyes are released due to the 5' nuclease activity of the Taq polymerase, resulting in an increased characteristic fluorescence of the reporter dye. Exonuclease activity only happens on the perfectly hybridized probes, since a probe containing a mismatched base will not be recognized by the Taq polymerase.

TCF7L2 genotyping:

Genomic DNA was extracted from blood samples using the Gentra Kit Method. Genotyping of TCF7L2 variants rs7903146, rs12255372, and rs4565650 were carried out by the 5′ nuclease assay using TaqMan® MGB probes on a Real Time PCR. (Applied Biosystems).The 5′ nuclease assay was performed in a 10 µl volume, comprising 15–25 ng genomic DNA, 1× TaqMan® Universal PCR Master Mix (Applied Biosystems), and 1× probe mix under the following amplification conditions: 10 min at 95°C and 40 cycles at 95°C for 15 s and 60°C for 1 min. Reference controls as well as non template controls were included in each run. Genotypes were automatically determined by Step One Pluse Software followed by a visual control of accurate genotype classification. TCF7L2 genotyping was carried out in a 96-well plate (Thermo scientific 96 well) using TaqMan allelic discrimination assay 1µl of extracted DNA was added to the 96-well plate and was analysed with the use Step One Plus Real Time PCR .(For every plate, there were three positive control sample and two samples of non-template controls). The results for these controls were the same for every analysis

- Genotyping of TCF7L2 variants rs7903146, rs12255372, and rs4506565 in 117 Gestation diabetes patients.

The Taqman Assay for SNP genotyping Procedure :

- **Step 1: Diluting SNP Genotyping Assays**
- **Step 2: Perform PCR amplification run:**
- **Step 3: Allelic Discrimination**

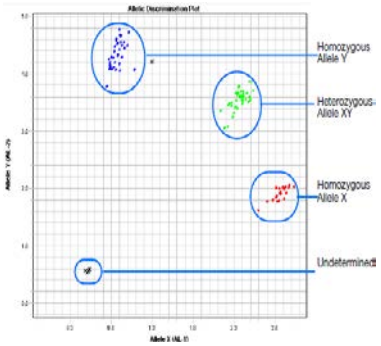
All genotyping included 5 - 10% duplicate samples to determine mistyping rates, which were 0%.

- Positive control

i. Heterozygous Positive(Allele 1 &Allele 2)
ii. Homozygous Positive(Allele 1 &Allele 1)
iii. Homozygous Positive (Allele 2 &Allele 2)

- Negative control

Samples Containing...	Are Grouped In...	Symbol
Allele X	Lower right corner of the plot	●
Allele Y	Upper left corner of the plot	●
Both (Allele X and Allele Y – heterozygote)	Approximately midway between the Allele X and Allele Y groups	●
No Template Control (NTC)	Bottom left corner of the plot (Not shown in the example.)	■
Undetermined	Anywhere on plot	X



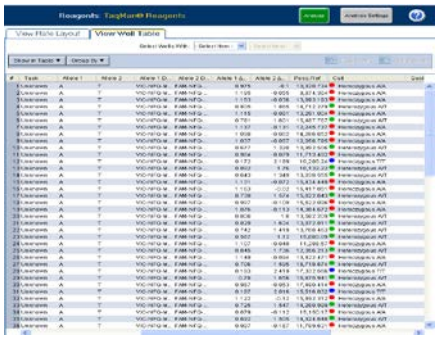


Figure-11A-Shows the grouping of all the three alleles (Wild type, Heterozygous and Homozygous); 11B- Scatter Plot showing allelic discrimination from the Step One Plus Software; 11-C shows the Variant call process in step one plus software

Statistical analysis:

Allele and genotype frequencies were calculated and consistency of genotype frequencies at each SNP with Hardy–Weinberg equilibrium was tested on a contingency table of observed and expected genotype frequencies using the using R statistical software. Further Genotype Frequencies were analysed using Cochran Armitage Trend Test with trend scores of 0,1,2 (0-wild, 1-hetero, 2-homozygous) and one degree of freedom using R statistical software. The allele and genotype frequencies between the GDM and the control subjects were compared using standard contingency table analysis. The clinical variables fasting and 2 hour post meal/OGTT glucose values were found to follow a non-normal distribution when analysed using Kolmogorov Smirnoff test. So we used a non-parametric test (Kruskal Wallis) to analyse the correlation between fasting and 2 hour post-meal glucose values with the genotypes of the three polymorphisms. Continuous variables were described using means and standard deviations (SD), if normally distributed. For variables with skewed distributions (eg: duration of diabetes), medians with interquartile ranges were used to summarize the distribution. All categorical variables were summarized by using frequencies and related percentages. The association between mutations, TCF7L2 polymorphisms and other covariates were tested by using ANOVA (3 categories), the independent two sample t-test or Chi-square test (2 categories), as appropriate. The data was analysed using SPSS version.16.0.

SECTION-I

A total of 50 subjects were screened for mutation in a panel of 10 MODY genes utilising a novel multiplex PCR protocol and sequencing was done on the next-generation sequencer (NGS), Ion torrent Personal Genome Machine. All the identified rare variants were confirmed by Sanger sequencing. Among the subjects who were screened16 (32%) had pre-gestational diabetes. Gestational diabetes was present in 18 (36%) subjects and 16 (32%) had overt diabetes (Figure12A&12B).

FIGURE-12A: DISTRIBUTION OF SUBJECTS BASED ON THE TYPE OF DIABETES

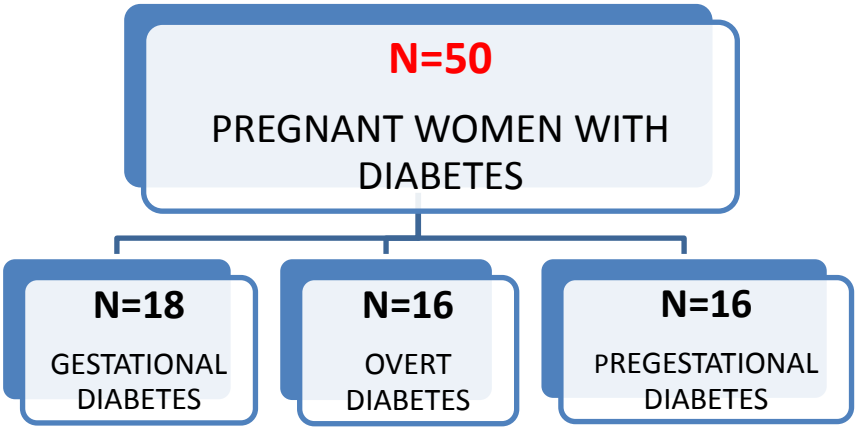


FIGURE-12B: PIE CHART DEPICTING THE DISTRIBUTION OF SUBJECTS BASED ON THE TYPE OF DIABETES. GDM: GESTATIONAL DIABETES, OVERT: OVERT DIABETES, PREGDM: PREGESTATIONAL DIABETES

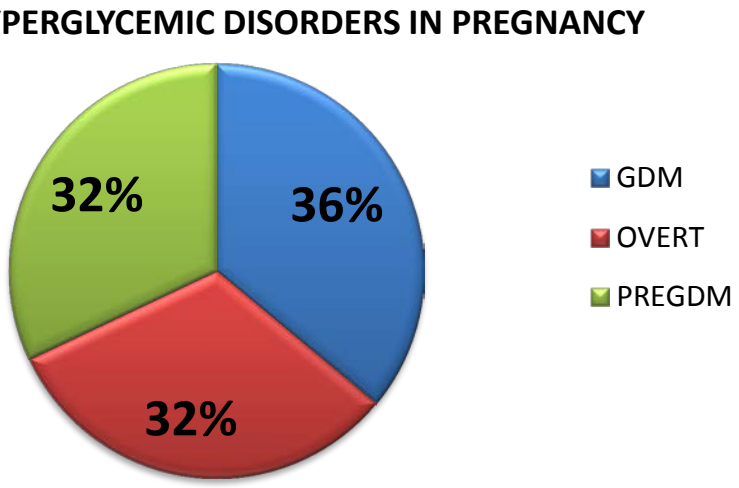


Table-7: BASELINE CHARACTERISTICS OF STUDY SUBJECTS

PARAMETERS	TOTAL [N = 50]	GDM [N = 18]	OVERT [N = 16]	PREGDM [N=16]	p-value
Age (years)mean ± SD	29.1 ± 5.1	28.2 ± 4.8	27.3 ± 4.8	31.4 ± 5.1	0.102
Age At Diagnosis (years) mean ± SD	28.3 ± 4.7	28.2± 4.8	27.7 ± 5.1	28.5 ± 6.8	0.905
Average Week of detection /Duration [Median & range]	-	20 weeks [8 to 32]	16 Weeks [5 to 32]	#22 months [1 to 60]	N/A
* BMI (kg/m2)	27.47 ± 3.82	26 ± 3.84	29.0 ± 2.56	26.4 ± 4.46	0.218
18.5 - 23	7 [14%]	2 (11.1%)	1(6.2%)	4(25%)	0.496
23.1-24.9	11[22%]	5(27.8%)	1(6.2%)	1 (6.2%)	
25-29.9	25 [50%]	7(38.9%)	10 (62.5%)	8(50%)	
30 – 34.9	7[14%]	4 (22.2%)	4(25%)	3(18.7%)	
3 generation family history of diabetes	27 [54%]	7 (11.1%)	9 (56.2%)	11 (68.8%)	0.213
Number of pregnancies					
Primigavida	24 (48%)	11 (61.1%)	7 (43.8%)	6 (37.5%)	0.357
Multigravida (≥ G2)	26 (52%)	7 (38.9%)	9 (56.2%)	10 (62.5%)	
Previous pregnancies					
Previous GDM	5 (20%)	2	1	1	N/S
Previous miscarriages	13 (50%)	6	5	2	
Previous congenital anomaly	1 (3.8%)	0	0	1	
Previous LGA	4 (15.4%)	1	1	2	
Previous LSCS	8 (30.7%)	3	1	4	
Glycaemic profile					
Fasting plasma glucose (mg%) [mean± sd]	125 ± 35	98±13	150±24	130 ± 38	<0.0001
2hour post meal Fasting plasma glucose (mg%) [mean± sd]	186 ± 58	147±23	213±51	206 ± 70	<0.003
HbA1c (%) [mean± sd]	7.18±1.51	5.5±0.5 [n=13]	7.4±1.2 [n=13]	8.2±1.3 [n=15]	<0.0001

*Calculated based on preconceptional weight / weight recorded at the visit in first trimester.
Except one subject who had diabetes for 16 years.
LGA / macrosomia=Birth weight of > 90th percentile (≥ 3.50 kg) in the neonates. BMI: Body Mass Index, N/S: not significant, N/A : not applicable. Multigravida (≥G2): a woman that is or has been pregnant for at least a second time. LSCS: Lower segment Caesarean section

The baseline characteristics of these subjects are summarised (Table.1).The mean age of all the subjects was 29.1 years, with subjects in the PREGDM group older (31.4 years) than those in the OVERT (27.3 years) and GDM (28.2 years) group. The median age of diagnosis of diabetes in the PREGDM group was 22 months excluding one subject (MG101) who had diabetes for 16 years [detected at the age of 10 years] and was negative for GAD antibodies. The clinical details of all the individual subjects are summarised in the master sheet. The median gestational week of diagnosis of OVERT and GDM subjects was 16 and 20 respectively. The mean BMI of the subjects in the study was 27.47kg/m^2, with the subjects in the OVERT group having a mean BMI (29kg/m^2) more than those in the GDM (26kg/m^2) and PREGDM group (26.4kg/m^2). Overall majority, 50% (n=25) subjects had a BMI between 25-29.9 kg/m^2, whereas 14% (n=7), 22% (n=11), and 14% (n=7) of the subjects had a BMI between 30-34.9, 23-24.9, and 18.5-23kg/m^2 respectively.

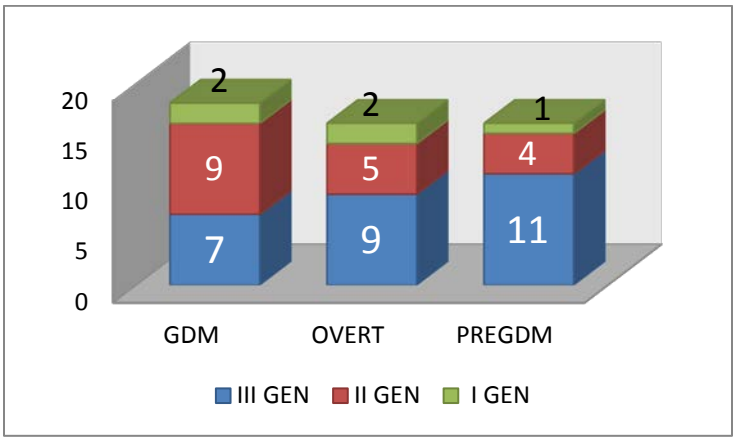
Forty eight percent (n=24) of the subjects were women in their first pregnancy (primigravida) and 52% (n=26) of the subjects were multigravida (≥ G2). The women with multigravida were more in the PREGDM (n=10) and OVERT (n=9) groups as compared to the GDM (n=7) group. In those women with multigravida, 5(20%) subjects had previously documented gestational diabetes, 13 (50%) had miscarriage, 8(30.7%) caesarean delivery. The mean birth weight of the previously delivered offspring (n=22) was 3114.4±485grams and 4 (18%) neonates each had low birth weight and macrosomia respectively. One neonate with very low birth weight (4%) was still born.

The mean fasting and 2 hour post meal glucose levels were significantly different among the groups and are summarised in the above table. The mean HbA1c of the subjects in PREGDM group (8.2%) was more than that of the OVERT (7.4%) group.

Table-8: DISTRIBUTION BASED ON THE NUMBER OF GENERATIONS WITH A FAMILY HISTORY OF DIABETES

No. of generations	Total [N=50]	GDM [N = 18]	OVERT [N = 16]	PRE GDM [N=16]
III	**27 [54%]**	7 (11.1%)	9 (56.2%)	11 (68.8%)
II	**18 [36%]**	9 (50%)	5 (31.2%)	4 (25.0%)
I	**5 [10%]**	2 (7%)	2 (12.5%)	1 (6.2%)

FIGURE-13: BAR CHART SHOWING THE DISTRIBUTION OF SUBJECTS BASED ON A FAMILY HISTORY OF DIABETES



Women were classified based on the number of generations with a family history of diabetes. Overall 54% (27) of the subjects had a 3 generation(GEN) family history of diabetes suggestive of an autosomal dominant pattern inheritance, with subjects in the PREGDM (n=11) and OVERT (n=9) group more than those in the GDM(n=7) group.

The outcome of the offspring of 46 subjects was available for the analysis, of whom 45 women had delivered in our institution and one had delivered elsewhere. The male: female ratio was 26:20. One subject with GDM had a twin delivery, 1 subject (M35) with OVERT diabetes had a still birth and 1 subject (M31) with PREGDM had a miscarriage. The mean birth weight of the offspring was 2860grams. The birth weight of the offspring of PREGDM group was lower (2670grams) than that of the GDM (2890grams) and OVERT (2840grams) group. Sixty five percent (n=31) of the offspring were of normal birth weight (NBW) and 20% (n=11) were low birth weight. NBW offspring were more in the GDM group when compared to the other 2 groups. There was a trend towards a higher prevalence (p=0.061) of LBW in the PREGDM (6/15) group off springs in comparison to the GDM group (3/14). Three (5%) offspring were macrosomic. The preterm offspring accounted for 24.1% (n=12) of the deliveries. Overall 48.8% (n=23) of the

offspring were delivered by caesarean section with 6(26%), 7(30%) and 10(44%) subjects in the GDM, OVERT and PREGDM group respectively.

TABLE-9: CHARACTERISTICS OF THE OFFSPRING

PARAMETERS	TOTAL [N = 50]	GDM [N = 18]	OVERT [N = 16]	PREGDM [N=16]	P-VALUE
INSTITUTIONAL DELIVERY	46	14**	16##	16***	NS
SEX RATIO [F:M]	20:26	5:10**	5:9	9:7	N/A
BIRTH WEIGHT (grams) [N=46]	2860 ± 510	2890 ± 380	2840 ± 520	2670 ± 620	0.482
NBW	31(65%)	13	10##	8	*0.393
LBW	11(20%)	2	3	6	#0.061
LGA	3 (5%)	0	2	1	N/A
PRETERM	12 (24.1%)	3	3	6	0.801
CAESAREAN BIRTHS	23 (48.8%)	7	6	10	0.198

NBW: Normal birth weight, LBW: low birth weight, LGA: Large for gestational age, CS: Caesarean section,
**1 Twin delivery
##1 subject (M35) with OVERT diabetes had a still birth
***1 subject (M31) with PREGDM had a miscarriage
*Comparing GDM with OVERT , #Comparing GDM with PREGDM
LGA / macrosomia=Birth weight of > 90th percentile (≥ 3.50 kg) in the neonates (1)Balaji V, Indian J EndocrinolMetab. 2011;15(3):187

TABLE-10: THE INDICATIONS FOR CAESAREAN SECTION ARE SUMMARISED

INDICATION FOR CAESAREAN SECTION	GDM	OVERT	PREGDM
Prior caesarean birth	1	1	4
Breech	1	0	2
Failed Induction	2	5	2
NRFS	2	0	1
Arrest of Descent	1	0	0
Unfavourable cervix	0	0	1

NRFS: Non Reassuring Foetal Status (foetal distress)

RESULTS: NEXT GENERATION SEQUENCING FOR MODY MUTATION

In the present study, 50 women of Asian Indian origin with diabetes complicating their pregnancy were screened for a comprehensive panel of ten MODY genes utilizing a novel multiplex PCR-based next generation sequencing on the Ion torrent PGM. The results of the data processed and summary analysis for identification of mutation on NGS are as follows

STEPS IN DATA PROCESSING AND ANALYSIS FOR IDENTIFICATION OF MUTATION ON NGS PLATFORM

•**Step 1:** Sequencing was performed on the Ion Torrent PGM utilizing ION.314 chip (3 samples per run)

•**Step 2:** Analysis of the sequence run showed that 96.6% of the target was sequenced adequately at a minimum of 20x coverage.

•**Step 3:** The obtained reads were mapped to the human reference genome (hg19) using Torrent Mapping Alignment Program (TMAP).

•**Step 4:** Base calling & quality assessment of raw data was done by Torrent suit software (version v2.0/v3.6.2) and data with #Phred score of Q20 were selected for variant calling.

•**Step 5:** Variant calling was done with the DNA star software (DNASTAR, Madison, USA). Analysis of the DNA samples with the human gene mutation database (HGMDR Professional 2012.4) yielded around ~40 significant variants from each subject. The identified non-synonymous coding variants were classified as reported or novel.Further, all the identified novel variants were filtered against * ENSEMBL and **The 1000 GENOMES database for known or benign variants.

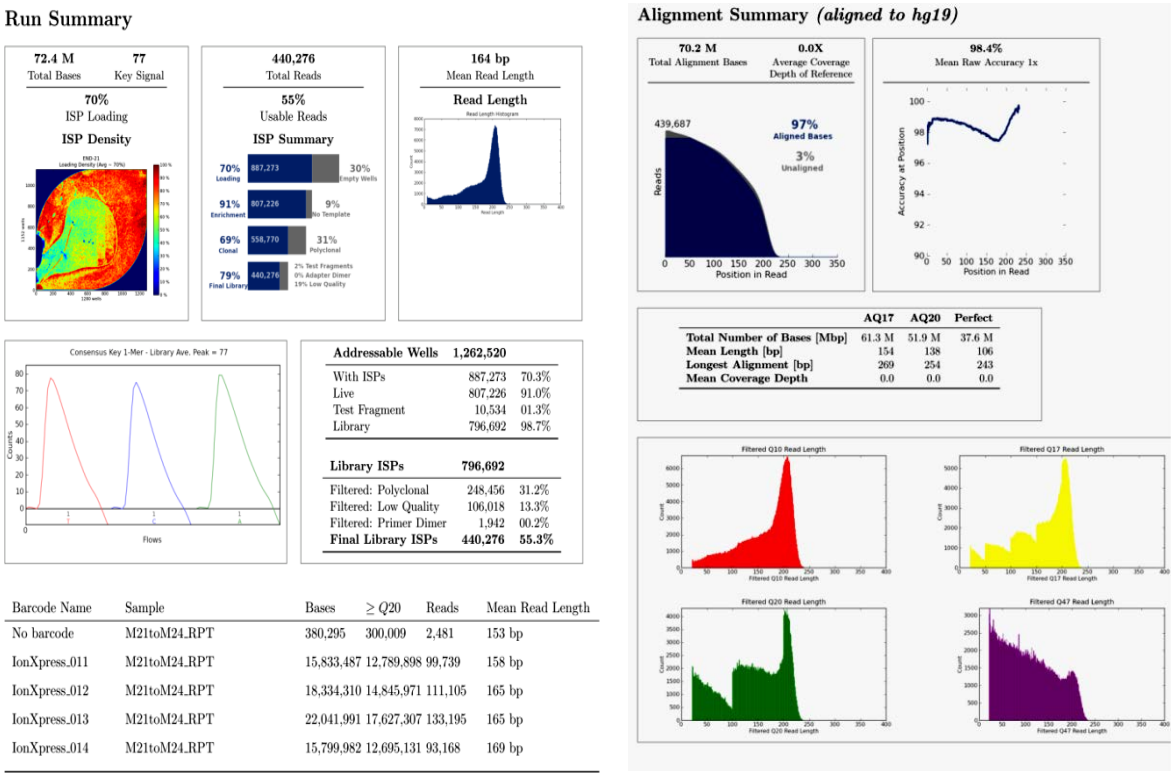
Step 6 & 7: All novel variants were evaluated for sequence conservation by genomic evolutionary rate profiling (GERP) and reported as probable pathogenic variant utilizing the bioinformatics predictive tools POLYPHEN-2, SORTING INTOLERANT FROM TOLERANT (SIFT) AND MUTATION TASTER. Subsequently, the pathogenic variants found in any of the 100 control alleles (subjects with normal glucose tolerance) were excluded.

Step 8: Validation of all the identified probable pathogenic variants was done by Sanger sequencing and then reported as mutation. (Figure 16B)

Phred score: Q20 indicates an accuracy of 99% or higher for the base called. Phred is a program that reads DNA sequencer trace data, calls bases and also assigns quality values to the bases, and writes the base calls and quality values to output files. *http://asia.ensembl.org, **http://www.1000genomes.org.

RESULTS – 2ND GENERATION SEQUENCING RUN/DATA REPORT

Figure-14: Ion Torrent PGM run report (314 chip), alignment summary after mapping tp hg19 and coverage analysis



Barcode Name	Sample	Bases	≥ Q20	Reads	Mean Read Length
No barcode	M21toM24_RPT	380,295	300,009	2,481	153 bp
IonXpress_011	M21toM24_RPT	15,833,487	12,789,898	99,739	158 bp
IonXpress_012	M21toM24_RPT	18,334,310	14,845,971	111,105	165 bp
IonXpress_013	M21toM24_RPT	22,041,991	17,627,307	133,195	165 bp
IonXpress_014	M21toM24_RPT	15,799,982	12,695,131	93,168	169 bp

coverageAnalysis

Barcode Summary Report

Barcode ID	Mapped Reads	On Target	Mean Depth	Uniformity	1x Coverage	20x Coverage	100x Coverage
IonXpress_011	96,502	86.42%	382.10	86.41%	98.389%	95.467%	82.458%
IonXpress_012	107,557	87.23%	447.06	86.74%	98.217%	95.934%	85.175%
IonXpress_013	128,130	82.43%	502.35	85.50%	98.250%	95.778%	85.503%
IonXpress_014	89,953	78.48%	343.89	85.77%	98.179%	94.435%	80.184%

variantCaller

Variant Caller Reports	Mapped Reads	Reads On-Target	Bases On-Target	Read Depth	1x Coverage	20x Coverage	100x Coverage	Variants Detected
IonXpress_011	98,957	84.51%	84.67%	382.73	98.394%	95.470%	82.600%	40
IonXpress_012	110,267	85.41%	85.45%	448.19	98.220%	95.934%	85.199%	51
IonXpress_013	132,122	80.17%	80.24%	503.19	98.253%	95.784%	85.527%	48
IonXpress_014	92,490	76.52%	76.28%	344.46	98.179%	94.441%	80.335%	51

ION TORRENT PGM RUN REPORT. Utilizing 200bp Ion torrent sequencing kit, with 70% loading, Ion PGM yielded an output of 51MB of Q20 data. 97% of the bases aligned to the reference genomehg19. We have multiplexed four samples which were sequenced at an average 419x mean depth coverage with >95% of the target sequenced at minimum coverage of 20x. Following the coverage analysis the Torrent variant caller was utilized to identify variant. This preliminary analysis was performed on Torrent suit software.

Figure-15: A prototype Ion torrent suit report showing that the average coverage depth of the entire targeted 10 genes sequenced at 452x, with >95% sequenced at 20x coverage.

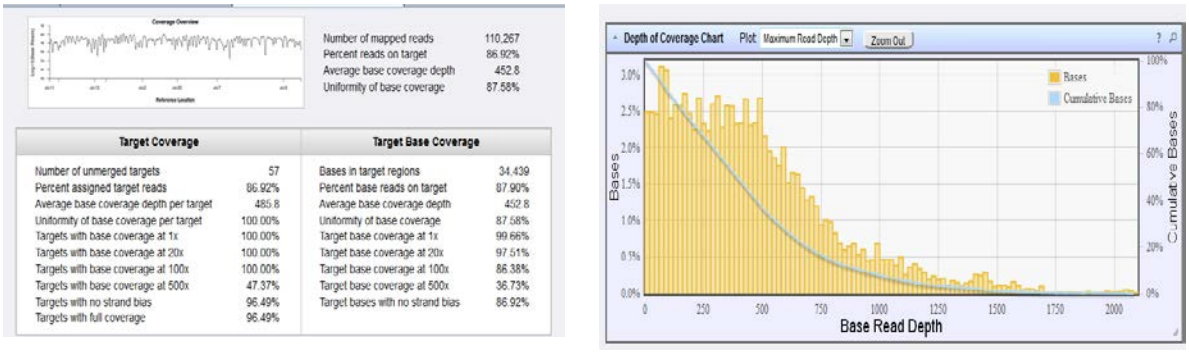


Figure-16: A report of nucleotide change identified by the next generation sequencing showing mutation in GCK and HNF1A gene in MG95 and MG53 respectively.

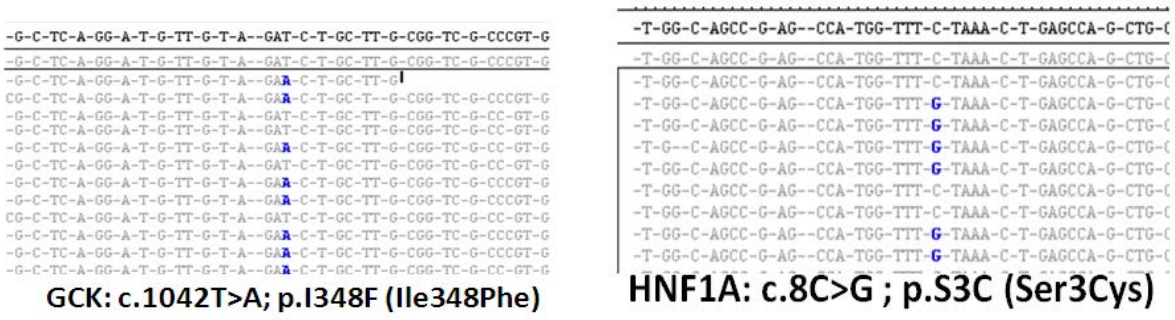


Figure-17: Report of predicting pathogenicity of novel variants [eg., HNF1A& GCK] by the bioinformatics tools POLYPHEN-2 &MUTATION TASTER.

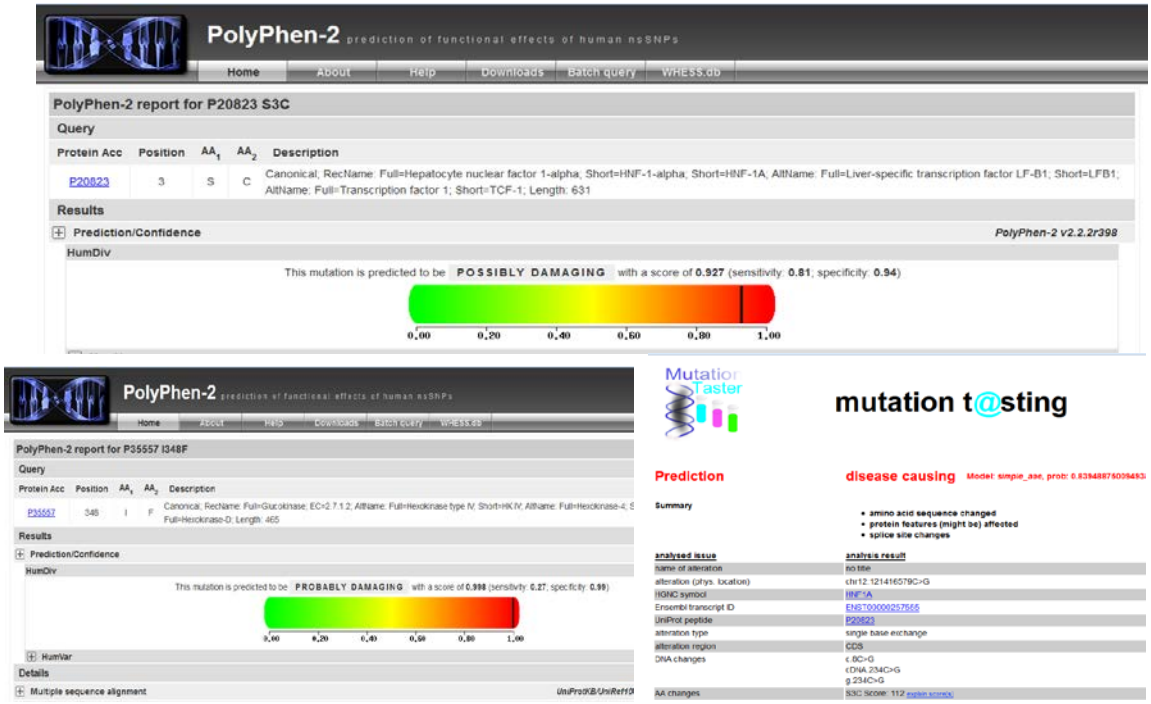
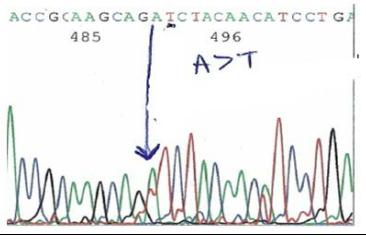
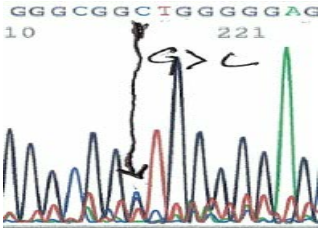
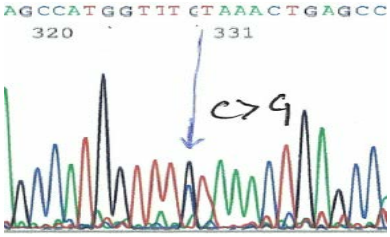
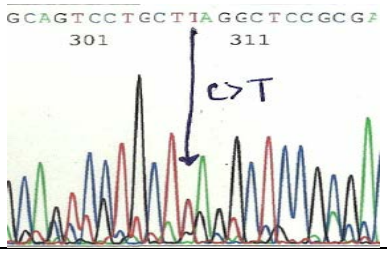
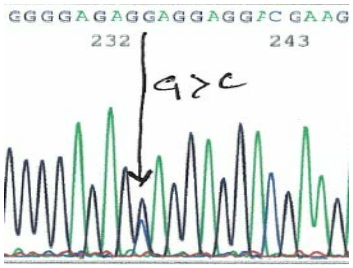
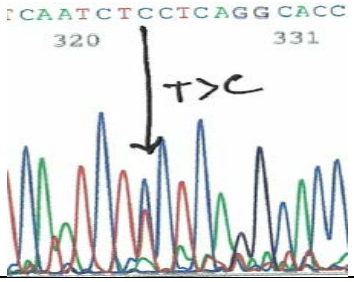
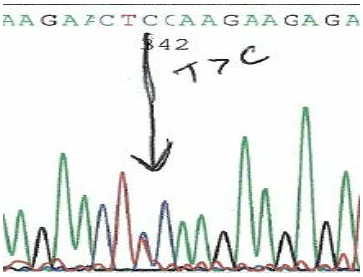
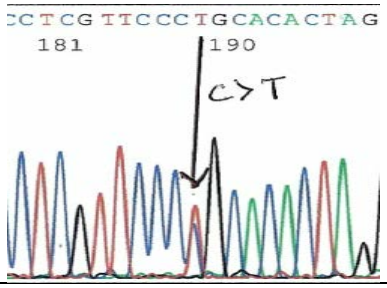


Figure 16B: ELECTROPHEROGRAM SHOWING SANGER CONFIRMATION OF THE IDENTIFIED MUTATIONS IN THIS STUDY.

MG95 MODY 2 	**MG53 MODY 3** 
GCKc.1042T>Ap.I348F Ile348Phe	HNF1A c.8G>C p.S3C Ser3Cys
MG19 MODY 4 	**MG101 MODY 4** 
PDX c.282C>G p.H94Q His94Gln	PDX c.670G<A p.E224K Glu224Lys
MG86 MODY 6 	**MG28 MODY 6** 
NEUROD1 c.953A>G p.E59Q Glu59Gln	NEUROD1 c.175C>G p.F318S Phe318Ser
MG102MODY 8* 	**MG42MODY 10** 
CEL c.248T>C p.F83S Phe83Ser	INS c.130C>T p.G44R Gly44Arg

Colour code: A-green, T-red, G-black , C-blue

TABLE-11: MUTATION RESULTS OF NEXT GENERATION SEQUENCING ON A PANEL OF 10 MODY GENES

MUTATION	GDM [N = 18]	OVERT [N = 16]	PRE GDM [N=16]
REPORTED *	NIL	NIL	1
NOVEL	1	4	2

- p.E224K Glu224Lys PDX1 mutation (subject: MG101)

Table-12: MUTATION DETAILS OF SUBJECTS WITH MODY

Sl. No	CODE	MODY	GENE	Nucleotide change	Aminoacid change	AOD	TYPE OF DIABETES	BMI (Kg/m2)	Baby birth weight {grams}
1	MG 95	MODY 2	GCK	c.1042 T>A	p.I348F Ile348Phe	23	OVERT	24	3140
2	MG 53	MODY 3	HNF1A	c.8C>G	p.S3C Ser3Cys	22	PREGDM	30	3940
3	MG 19	MODY 4	PDX	c.282C >G	p.H94Q His94Gln	35	OVERT	27	3300
4	MG 101	MODY 4	PDX	c.670G <A	p.E224K Glu224Lys	10	PREGDM	23	2240
5	MG 86	MODY 6	NEUROD1	c.953A >G	p.E59Q Glu59Gln	36	OVERT	30	3700
6	MG 28	MODY 6	NEUROD1	c.175C >G	p.F318S Phe318Ser	27	PREGDM	30	3100
7	MG 102	MODY 8*	CEL	c.248T >C	p.F83S Phe83Ser	28	GDM	28	2800
8	MG 42	MODY 10	INS	c.130C >T	p.G44R Gly44Arg	35	OVERT	27	2870

AOD: Age of diagnosis

We have identified MODY mutations in 16% (8/50) of the subjects. The mutation details are summarised in table 5. A higher frequency of NEUROD1 and PDX1 (two each) were mutations identified. The other mutations were one each in HNF1A, GCK, CEL and INS genes. Seven of the eight mutations were novel. The subject MG101 had a reported PDX1 mutation. Though, overall there was no significant difference among the subjects w.r.t age at diagnosis, BMI and birth weight of offspring, subjects with NEUROD1 mutation had a higher BMI(31.1kg/m^2) than those with other mutations(27kg//m^2). All except two subjects (MG101 with the reported E224K PDX1 mutation and MG53 with S3C HNF1A mutation) are currently managed with diet, metformin or sulphonylurea (SU) therapy.

Table-13: COMPARISON BETWEEN MODY POSITIVE AND NEGATIVE SUBJECTS

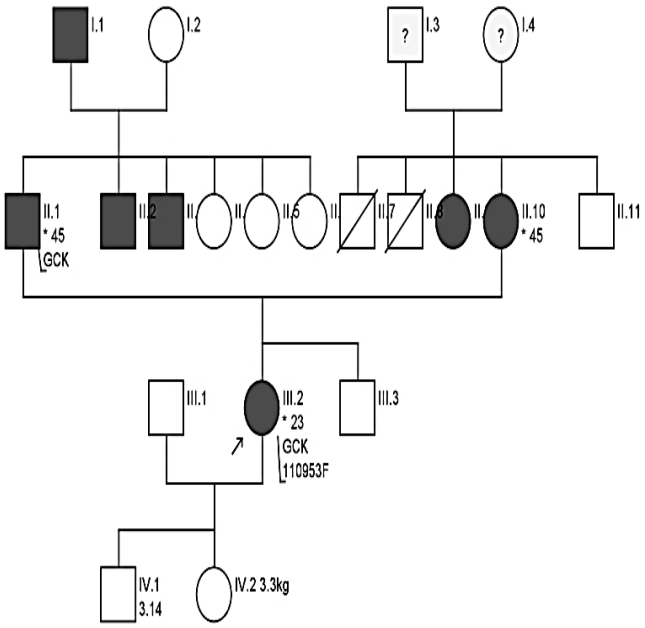
PARAMETERS	MODY POSITIVE [N = 8]	MODY NEGATIVE [N = 42]	P-VALUE
AGE (years) mean ± SD	**29.7 ± 5.3**	**28.9 ± 5.1**	0.722
AGE AT DIAGNOSIS (years) mean ± SD	**27.18 ± 3.67**	**28.33 ± 4.69**	0.748
HEIGHT (cms) mean ± SD	**158.25 ± 3.57**	**154.82 ± 6.47**	0.048
WEIGHT (kgs) mean ± SD	**70.88 ± 11.31**	**65.45 ± 11.95**	0.494
*** BMI (kg/m2) mean ± SD**	**28.19 ± 3.67**	**27.18 ± 3.70**	0.483
Primigavida **Multigravida (≥G2)**	**2** **6**	**22** **20**	**0.082**
GDM **OVERT** **PREGDM**	**1** **4** **3**	**17** **12** **13**	**0.017**
Generations with family history of DM **3** **≤ 2**	 **7** **1**	 **20** **22**	**0.038** **OR 1.291 [CI 1.016-1.641]**
BABY BIRTH WEIGHT (grams)	**3136 ± 531**	**2733 ± 484** [N=38]	0.078
NORMAL DELIVERY	**4**	**19**	**N/A**
CAESAREAN BIRTHS	**4**	**19**	
PRETERM	**1**	**11**	
LBW	**1**	**10**	
NBW	**5**	**24**	
LGA	**2**	**4**	
M:F	**3:5**	**23:15**	

In this study, utilising the Next generation sequencing approach we have identified MODY mutations in 16% (8/50) of subjects. Pregnant women with overt and pregestational diabetes (7/32) had a higher prevalence of mutation in comparison with those with gestational diabetes (1/18). There was significantly increased predisposition in MODY mutation positivity (p=0.017) in subjects with overt and pregestational diabetes in comparison with those with GDM. Seven (26%) of the 27 subjects with a 3 generation family history of diabetes were positive for MODY mutation, whereas only one (4%) of the 23 subjects without a 3 generation family history of diabetes was positive for MODY mutation. The MODY positivity was significantly high (p= 0.038) in those with a 3 generation family history diabetes with odds ratio (OR) of 1.291[CI=1.016-1.641].

GPC I: GENE: MODY 2: GLUCOKINASE (GCK): CHROMOSOME 7p13

MUTATION: I348F Ile348Phe [NOVEL]

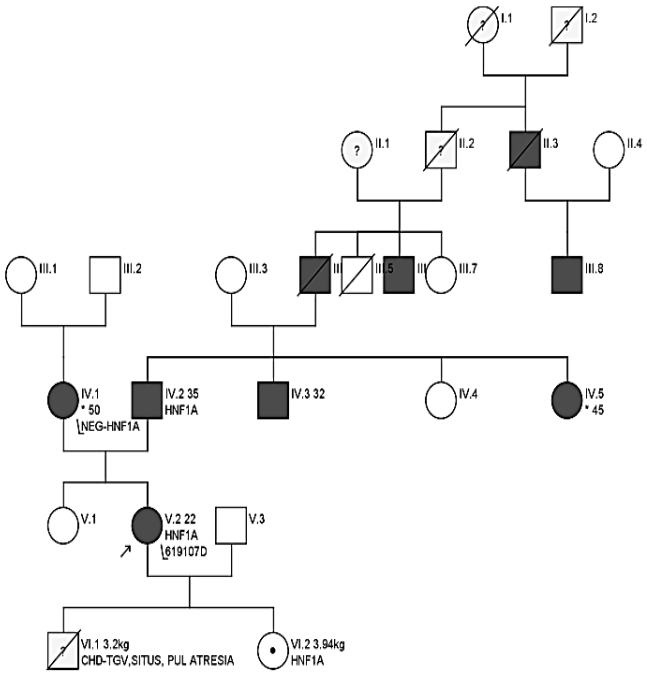
SUBJECT CODE NO: MG95

Age:	23 years
Diagnosis:	OVERT DIABETES MELLITUS
Age at diagnosis	23 years
Gestational age at diagnosis	21 weeks
Obstetric Score:	Gravida 2 Para 1 Living 1
BMI:	24kg/m^2
Current pregnancy: Birth details	Full term normal institutional delivery [FTND]. No neonatal hypoglycemia or other complications. Female baby with birth weight 3300 grams.
Previous Pregnancy	**GDM:** Yes, managed with insulin. **G1**: FTND. Male baby, birth weight: 3140 grams
75gram Oral GTT {mg/dl} **HbA1c (**%) Management during pregnancy	{0hr: 123, 1hr:229, 2hr: 201} 7 Insulin
Number of generations with family history of Diabetes Mellitus & Family tree **[Proband : III.2]**	3 
Family Members tested for GCK	Father: positive, Mother: negative
Post-delivery glycemic profile Fasting, 2hr postmeal glucose	92, 126{mg/dl}
Post-delivery therapy	Dietary modification

GPC II: GENE:MODY3:HEPATOCYTE NUCLEAR FACTOR-1-ALPHA(HNF1A):Chromosome 12q24.2

MUTATION: S3C:Ser3Cys [NOVEL]

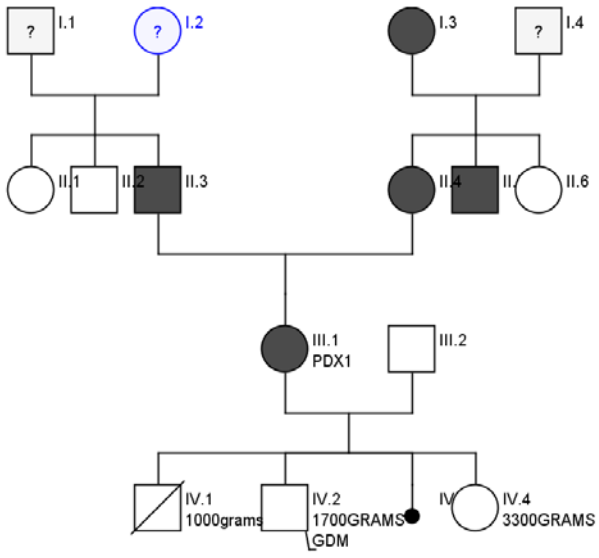
SUBJECT CODE NO: MG53

Age:	25 years
Diagnosis:	PREGESTATIONAL DIABETES MELLITUS
Age at diagnosis	22 years
Obstetric Score:	Gravida 2 Para 1 Living 0, Neonatal death 1
BMI:	30kg/m^2
Current pregnancy: **Birth details**	Full tern normal delivery [FTND]. No neonatal hypoglycemia or other complications. Female baby with birth weight 3940 grams
Previous Pregnancy	**PREGDM:** Yes, managed with insulin. **G1**:FTND. Male baby, birth weight: 3340 grams Expired at the age of 1 month due to congenital heart disease: Transposition of great vessels with pulmonary atresia
75gram Oral GTT {mg/dl} **HbA1c (**%) Management during pregnancy	{0hr: 163, 1hr:272, 2hr: 288} 6.4 Insulin
Number of generations with family history of Diabetes Mellitus & Family tree **[Proband : V.2]**	3+ 
Family members tested for HNF1A	Father : positive, Mother: negative, current child: positive
Post-delivery glycemic profile Fasting, 2hr postmeal glucose {mg/dl}& HbA1c	174,195&9.5%
Post-delivery therapy	Metformin and Sulphonylurea. Insulin was added for good glycemic control as she was planning for conception.

GPC III: GENE: MODY 4:PANCREAS/DUODENUM HOMEOBOX PROTEIN-1 (PDX1): Chr:13Q12.1

MUTATION: H94Q His94Gln [NOVEL]

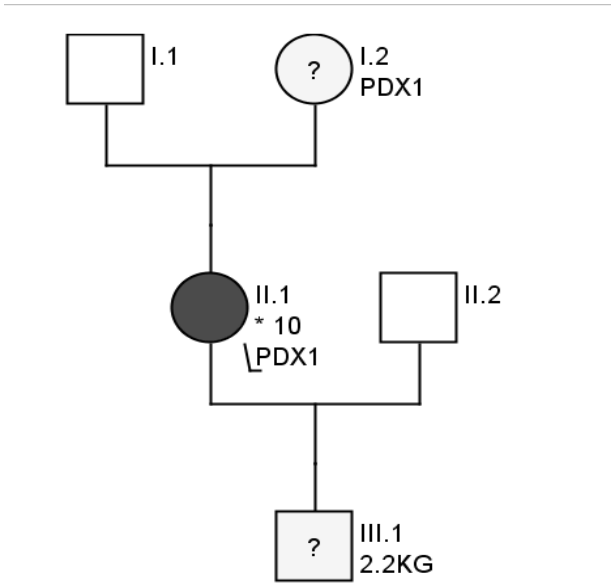
SUBJECT CODE NO: MG19

Age:	35 years
Diagnosis:	OVERT DIABETES MELLITUS
Age at diagnosis	35 years
Gestational age at diagnosis	5 weeks
Obstetric Score:	Gravida 4 Para 2 Living 1 Neonatal death 1 Abortion1
BMI:	27kg/m^2
Current pregnancy: Birth details	Preterm [36wks] institutional delivery by caesarean section. No neonatal hypoglycemia or other complications. Male baby with birth weight 3300 grams. Gestational hypertension+
Previous Pregnancy	**GDM: YES,** G1- Caesarean delivery -32 weeks [preterm]; Birth weight: 1000 grams, neonatal death, G2 – miscarriage G3- Caesarean delivery -36 weeks Birth weight 1700 grams
Fasting, 2hr postmeal glucose {mg/dl} **HbA1c (**%) Management during pregnancy	{0hr: 172, 2hr: 226} 6.4 Insulin
Number of generations with family history of Diabetes Mellitus & Family tree **[Proband : III.2]**	2 
Family Members tested for PDX1	none
Post-delivery glycemic profile Fasting, 2hr postmeal glucose {mg/dl}	129, 206
Post-delivery therapy	Metformin

GPC IV: GENE: MODY 4:PANCREAS/DUODENUM HOMEOBOX PROTEIN-1 (PDX1): Chr:13Q12.1

MUTATION: E224K, Glu224Lys [REPORTED]

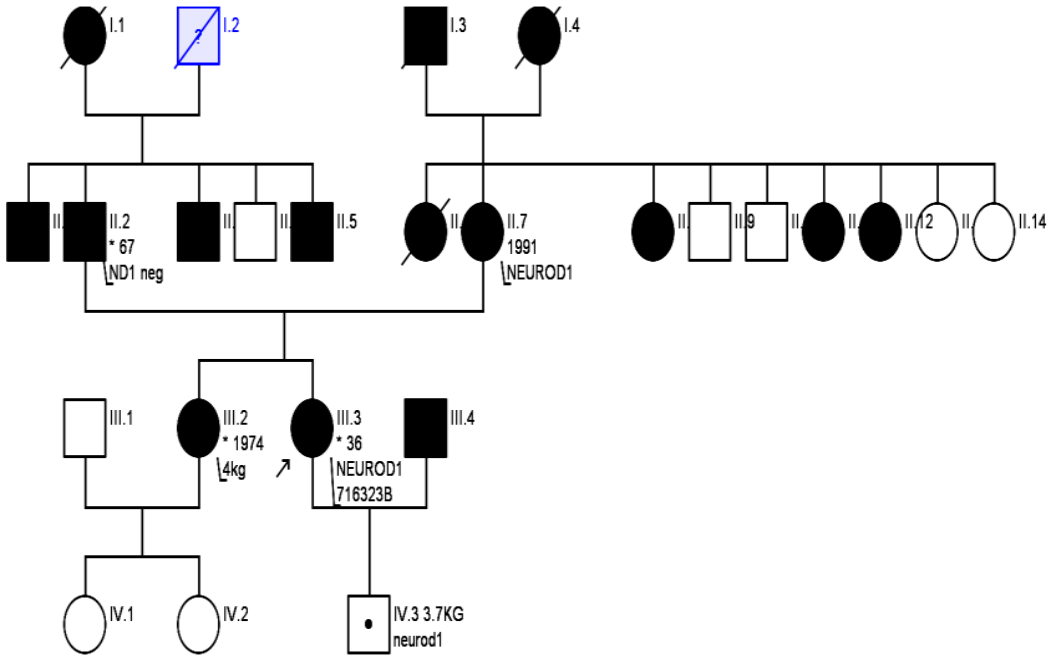
SUBJECT CODE NO: MG101

Age:	25 years
Diagnosis:	PREGESTATIONAL DIABETES MELLITUS
Age at diagnosis	10 years
Obstetric Score:	Primigravida
BMI:	23 kg/m^2
Current pregnancy: **Birth details**	Preterm [36 weeks] vaginal delivery. No neonatal hypoglycemia or other complications. Male baby with birth weight 2240 grams
Previous Pregnancy	**N/A**
Fasting, 2hr postmeal glucose {mg/dl} **HbA1c (**%) Management during pregnancy	{53, 153} 7.2 Insulin
Number of generations with family history of Diabetes Mellitus & Family tree **[Proband: II.1]**	1 
Family Members tested for PDX1	Mother : positive , Father : Negative.
Post-delivery glycemic profile HbA1c:	8.8%
Post-delivery therapy	INSULIN. She had ketoacidosis at onset. She has no features of pancreatic exocrine insufficiency

GPC V: GENE: MODY 6: NEUROGENIC DIFFERENTIATION 1 (NEUROD1): chromosome 2q32

MUTATION: E59Q Glu59Gln [NOVEL]

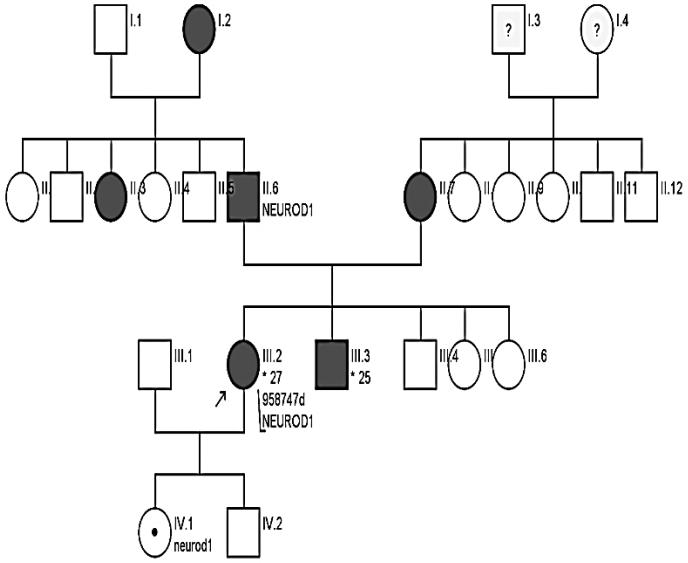
SUBJECT CODE NO: MG86

Age:	36 years
Diagnosis:	OVERT DIABETES MELLITUS
Age at diagnosis	36 years
Gestational age at diagnosis	24 weeks
Obstetric Score:	Primigravida
BMI:	32.4 kg/m^2
Current pregnancy: **Birth details**	Full term institutional delivery by caesarean section. No neonatal hypoglycemia or other complications. Male baby with birth weight 3700 grams
Previous Pregnancy	N/A
75gram Oral GTT {mg/dl} **HbA1c (**%) Management during pregnancy	{0hr: 119, 1hr:172, 2hr: 229} Insulin
Number of generations with family history of Diabetes Mellitus = 3 **Family tree[Proband : III.3]**  	
Family Members tested	Mother : positive , Father : Negative, Sister: positive, Son: positive
Post-delivery glycemic profile	0hr:191mg/dl, 2hr: 262mg/dl
Post-delivery therapy	Metformin with Glipizide

GPC VI: GENE: MODY 6: NEUROGENIC DIFFERENTIATION 1 (NEUROD1): chromosome 2q32

MUTATION: F318S Phe318Ser [NOVEL]

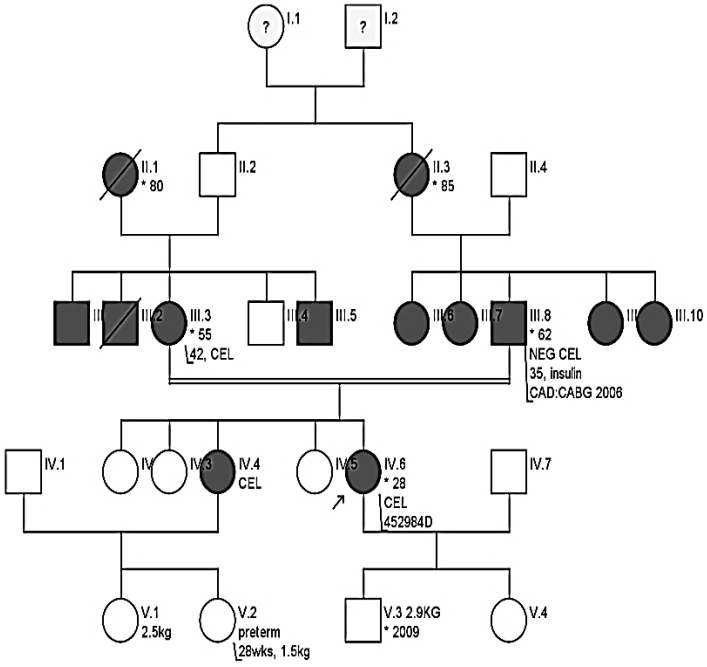
SUBJECT CODE NO: MG28

Age:	27 years
Diagnosis:	PREGESTATIONAL DIABETES MELLITUS
Age at diagnosis	25 years
Obstetric Score:	Gravida 2 Para 1 living 1
BMI:	30 kg/m^2
Current pregnancy: **Birth details**	Full term institutional delivery by caesarean section. No neonatal hypoglycemia or other complications. Female baby with birth weight 3100 grams
Previous Pregnancy	GDM: yes G1: Premature rupture of membranes, 36 weeks ,Caesarean institutional delivery. Male,Birth weight 2020grams , alive and well.
Fasting, 2hr postmeal glucose {mg/dl} **HbA1c (**%) Management during pregnancy	{92,162} 7.7 Insulin
Number of generations with family history of Diabetes Mellitus & Family tree **[Proband : III.2]**	3 
Family Members tested for NeuroD1	Mother : negative , Father : Positive, Son: negative, Daughter: positive
Post-delivery glycemic profile	Fasting, 2hr postmeal glucose {mg/dl} {132,200} HbA1c 6.8%
Post-delivery therapy	Metformin + Glimepiride
OTHER ILLNESS	Primary Hypothyroidism

GPC VII: GENE: MODY 8: CARBOXYESTER LIPASE (CEL) (Bile salt dependent lipase) chromosome 9q34

MUTATION: F318S Phe318Ser [NOVEL]

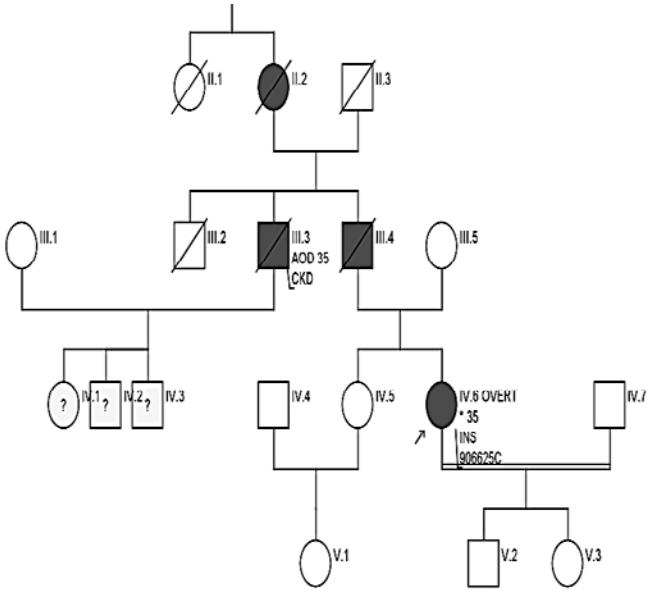
SUBJECT CODE NO: MG102

Age:	28 years
Diagnosis:	GESTATIONAL DIABETES MELLITUS
Age at diagnosis	25
Obstetric Score:	Gravida 4 Para 1 living 1 MTP 2
BMI:	28 kg/m^2
Current pregnancy: **Birth details**	Normal full term Caeserianextra-institutional delivery. No neonatal hypoglycemia or other complications. Female baby with birth weight 2800 grams
Previous Pregnancy	GDM: NO G1: 36 weeks ,Caeserian institutional delivery. Male, Birth weight 2900grams , alive and well.
Fasting, 2hr postmeal glucose {mg/dl} **HbA1c (**%) Management during pregnancy	{99, 147} 5.6 Insulin
Number of generations with family history of Diabetes Mellitus & Family tree **[Proband : IV.6]**	3 
Family Members tested	Mother : negative , Father : Positive, Sister : positive
Post-delivery glycemic profile	Data not available
Post-delivery therapy	Data not available

GPCVIII: GENE: MODY 10: INSULIN GENE (INS) ON CHROMOSOME 11P15.5.

MUTATION: G44R Gly44Arg [NOVEL]

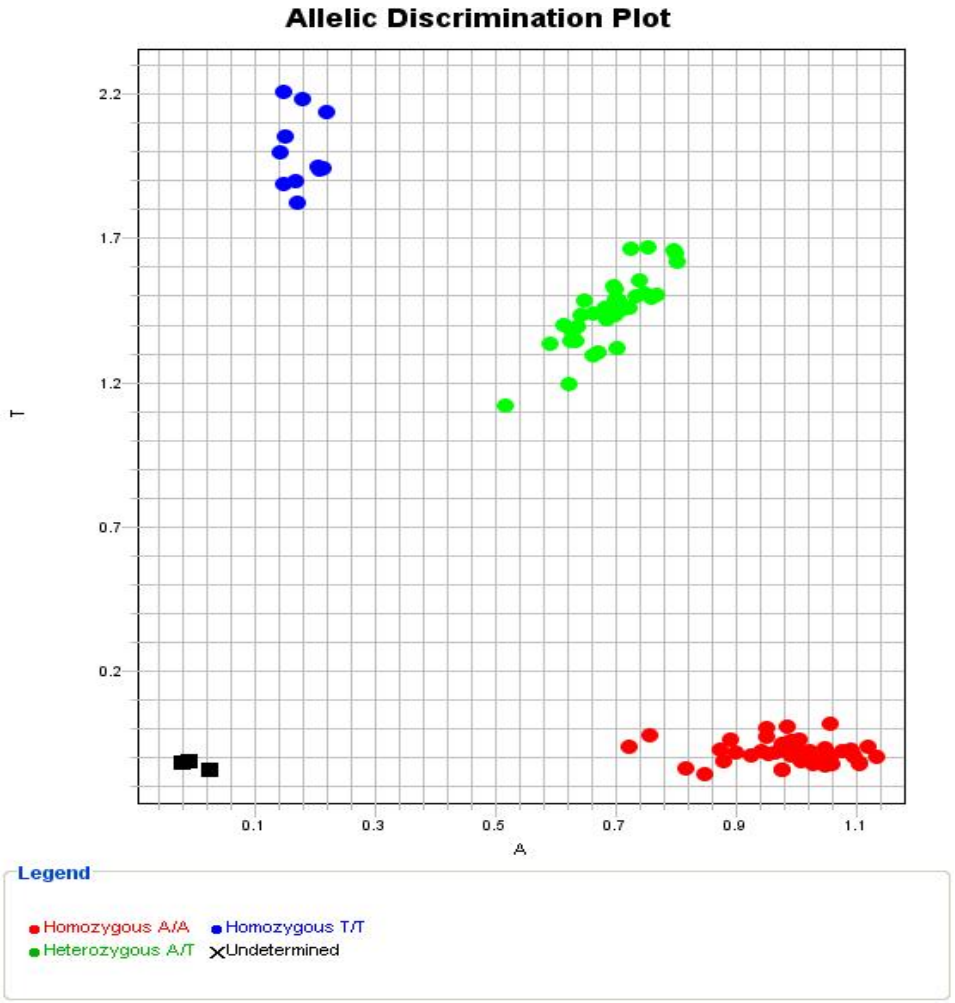
SUBJECT CODE NO: MG42

Age:	35 years
Diagnosis:	OVERT DIABETES MELLITUS
Age at diagnosis	35
Gestational age at diagnosis	28 WEEKS
Obstetric Score:	Gravida 2 Para 1 living 1
BMI:	27 kg/m^2
Current pregnancy: **Birth details**	Normal full term vaginal extra-institutional delivery. No neonatal hypoglycemia or other complications. Female baby with birth weight 2870 grams
Previous Pregnancy	Previous GDM: NO G1: 38weeks , institutional delivery. Male, Birth weight
75gram Oral GTT {mg/dl} **HbA1c (**%) Management during pregnancy	{0hr: 119, 1hr:172, 2hr: 229} 7.3 Insulin
Number of generations with family history of Diabetes Mellitus & Family tree **[Proband : IV.3]**	3 
Family Members tested	Son: Negative, Daughter: negative
Post-delivery glycemic profile	83, 169 mg/dl
Post-delivery therapy	DIET

SECTION-II TCF7L2 and Gestational diabetes

The genotyped 3 TCF7L2 polymorphisms (rs7903146, rs12255372 and rs4506565) using a TaqMan allelic discrimination assay revealed the following allelic discrimination plot signifying that the Genotype calls are 100% accurate.

ALLELIC DISCRIMINATION PLOT:



SNP Genotyping using Taqman Assays was done in 117 samples for all the three polymorphisms and the plots were intact signifying that the Genotype calls are 100% accurate.

Genotype and allele frequencies were analysed for Hardy Weinberg Equilibrium using R statistical software and found to be within Hardy Weinberg Equilibrium (Table 14).

TABLE-14 GENOTYPE AND ALLELE FREQUENCIES ANALYSED FOR HARDY WEINBERG EQUILIBRIUM

Polymorphism	Allele 1	Allele 2	Minor Allele	Major Allele	Chi-square Value	p-value
rs7903146	C	T	0.315	0.685	1.35	0.245
rs12255372	G	T	0.219	0.781	0.591	0.441
rs4506565	A	T	0.319	0.681	1.187	0.275

Genotype Frequencies were analysed using Cochran Armitage Trend Test with trend scores of 0,1,2(0-wild, 1-hetero, 2-homozygous) and one degree of freedom using R statistical software. The results showed no statistical significance towards the onncurance for any particular genotype (Table-15).

TABLE-15 TREND TEST RESULT WITH TREND SCORES OF 0,1,2 (0-WILD, 1-HETERO, 2 HOMOZYGOUS)

EXPOSURE		Outcome		Cochran Armitage Trend Test		
		Diseased	Controls	X^2	df	p value
SNP1	Wild	16	4	1.118	1	0.2917
	Hetero	46	18			
	Homo	55	26			
SNP2	Wild	8	2	0.7508	1	0.3862
	Hetero	38	14			
	Homo	70	32			
SNP3	Wild	17	3	2.5477	1	0.1105
	Hetero	47	17			
	Homo	53	26			

TABLE-16: CORRELATION BETWEEN **FASTING PLASMA GLUCOSE AND 2 HOUR POST-MEAL GLUCOSE** VALUES WITH THE GENOTYPES OF THE THREE POLYMORPHISMS**.**

		rs7903146			rs12255372			rs4506565		
		Wild	*Hetero*	*Homo*	*Wild*	*Hetero*	*Homo*	*Wild*	*Hetero*	*Homo*
Fasting plasma Glucose	N	39	40	13	53	30	8	37	41	14
	Mean	118.62	117.75	131.15	118.43	123.87	118.13	116.08	120.56	128.79
	SD	34.705	29.906	39.696	32.406	34.571	40.162	32.057	32.737	39.155
	Median	105.00	108.00	125.00	108	117	111.5	105	112	120.5
	p value	0.39			0.782			0.435		
Post prandial plasma Glucose	N	36	34	11	48	26	7	34	35	12
	Mean	178.75	171.59	226.00	180.02	183.04	193.57	176.24	176.71	214.83
	SD	80.458	65.657	94.524	73.848	82.551	96.35	77.77	69.26	98.076
	Median	162.50	152.50	207.00	166	156	196	162.5	155	203.5
	p value *	0.167			0.905			0.399		

The clinical variables fasting and 2 hour post meal/OGTT glucose values were found to follow a non-normal distribution when analysed using Kolmogorov Smirnoff test. So we used a non-parametric test (Kruskal Wallis) to analyse the correlation between fasting and 2 hour post meal/OGTT with the genotypes of the three polymorphisms. Among the 3 SNPs, the results were found to be statistical non significant

TABLE-17: GENOTYPE FREQUENCIES AND ESTIMATES OF RELATIVE RISKS OF TCF7L2 VARIANTS IN GESTATIONAL DIABETES SUBJECTS AND CONTROL SUBJECTS (ANOVA)

SNP	Geno type	Patients (n=117)	Control subjects (n=49)	Hetero OR (95% CI)	P-Value	Homo OR (95% CI)	P-value
rs7903146	CC	55	27	1.184(0.551-2.544)	0.666	1.776(0.503-6.263)	0.37
	CT	46	18				
	TT	16	4				
rs12255372	GG	70	33	1.354(0.618-2.966)	0.449	1.404(0.242-8.119)	0.705
	GT	38	14				
	TT	8	2				
rs4506565	AA	53	27	1.411(0.652-3.05)	0.382	3.75(0.750-18.53)	**0.08**
	AT	47	17				
	TT	17	2				

n = number of individuals, Genotype relative risk (GRR) was calculated compared with the baseline genotype (homozygote for the common allele). Hetero Odds ratio [OR] GRR for heterozygotes;Homo OR GRR for homozygotes

In this study we have included all the three TCF7L2 (SNPs) i.e. (rs7903146, rs12255372 and rs4506565) in GDM separately, excluding overt and pre-gestational diabetes in south Indian population and the results were rs7903146 (odds ratio [OR] 1.77 [95% CI=0.50-6.26], p=0.370), rs12255372 (OR 1.40 [95% CI= 0.24–8.11], p=0.705) and rs4506565 (OR 3.75 [95% CI=0.75-18.53],p=0.08). In our population we have found that the polymorphism rs4506565 showed a strong trend towards association [O.R=3.75(C.I=0.75-18.53) p=0.08] in comparison to the other two polymorphisms (rs7903146, rs12255372).

SECTION-I:

In the present study, 50 women with diabetes complicating their pregnancy were screened for a comprehensive panel of ten MODY genes utilizing multiplex PCR-based next generation sequencing on the Ion torrent PGM. The mean age of the subjects was 29 years, with most of them (68%, n=34) above the age of 25 years. The mean body mass index (BMI) was 27.5kg/m^2 and more than 75% of the pregnant women had first degree relatives with diabetes. As has been observed in the present study, a community based survery(151) from a similar population from the state of Tamil Nadu that an age > 25 years, BMI > 25kg/m2 and the presence of family history of diabetes had significant independent association with GDM (P<0.001). There was no significant difference in the mean age within the three groups viz., pregnant women with pregestational, overt or gestational diabetes, though the mean age of women with pregestational diabetes was higher (31 vs 28 years) and had a higher parity (62% vs 53%) in comparison to the other two groups. Similar findings have been noted in the recent studies by Fong et al(152), Wong et al (59) and Sugiyama et al (60) where the mean age were not significantly different within the groups, and in the study by Fong et al(152) women with pregestational diabetes were older with increased parity.

In the current study, those with overt diabetes in pregnancy in comparison to women with gestational diabetes were diagnosed earlier in pregnancy(16 vs 20 weeks), had a higher antenatal HbA1c(7.4 vs 5.5%) and pre-pregnancy BMI (29 vs 26kg/m2) which is similar to the recent reports by Wong et al (59) and Sugiyama et al (60).

Caesarean deliveries were more in the pregestational group in comparison to the women in the other groups (62.5% vs 44%) , similar to that reported by Fong et al(152). As noted by Sugiyama et al(60), we also found that macrosomia was more in the

offspring of pregnant women with overt diabetes in comparison to that of the GDM group (2 vs 0).

Postpartum, of the seven patients with overt diabetes in pregnancy (n=16) who underwent a follow-up oral glucose tolerance test and/or HbA1c at 12 weeks post-partum, more than 50% (n=4) had either diabetes or impaired glucose tolerance, whilst others returned to normal glucose tolerance which is similar to the study by Wong et al (59) where about 27% had diabetes and 33% had impaired glucose tolerance with the rest 40% returning to normal glucose tolerance. Hence, women with overt diabetes in pregnancy are more likely to have underlying beta-cell dysfunction prior to becoming pregnant in comparison to subjects with mild gestational diabetes. They are also more likely to have persistent glucose intolerance post-partum and hence need to be carefully monitored during and after pregnancy. Not many studies have been reported from India after the introduction of the new classification of hyperglycemic disorders in pregnancy by International Association of Diabetes and Pregnancy Study Groups (IADPSG) consensus panel in 2010 (145). Most of the earlier studies on gestational diabetes have either looked at gestational or pregestational diabetes separately and have used various criteria (WHO, DIPSI, ADA, ACOG etc.,) for classification of gestational diabetes.

Next generation sequencing (NGS):

Utilizing multiplex PCR-based target enrichment coupled with the Ion Torrent NGS, we screened a comprehensive panel of ten MODY genes in 50 pregnant women with diabetes. The samples were sequenced at an average depth of 501x and 96.6% of the target was sequenced at a minimum coverage of 20x. In this study mutations were identified in eight (16%) patients, seven of which were novel. These novel mutations were absent in 100 control alleles. Till date we have not found any literature that has looked at a comprehensive panel of 10 MODY genes in pregnant women with diabetes.

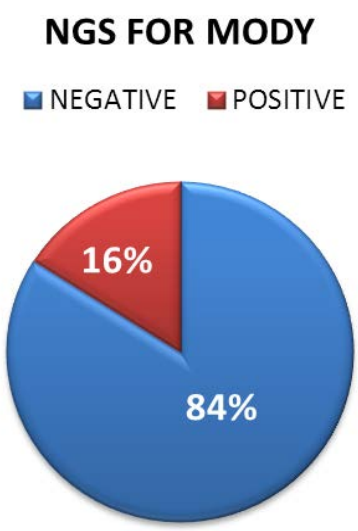
In a recent study utilising a panel of 29 genes implicated in monogenic diabetes, a genetic diagnosis of MODY was obtained in 15% (5/33) and of neonatal diabetes in 18% (9/49) of subjects. They have also shown that the NGS approach is superior and highly sensitive (153). In a similar study done at our centre (unpublished data) a novel multiplex polymerase chain reaction (PCR) based target enrichment followed by NGS on the Ion Torrent Personal Genome Machine (PGM) was utilised to identify mutations in a comprehensive panel of ten MODY genes. In the study, 56 out of the 80 subjects of Asian-Indian origin with young onset diabetes were clinically(77) diagnosed as MODY. Mutations were identified in 19% (11/56) of the clinically diagnosed MODY subjects with a higher frequency of mutation in NEUROD1 gene.

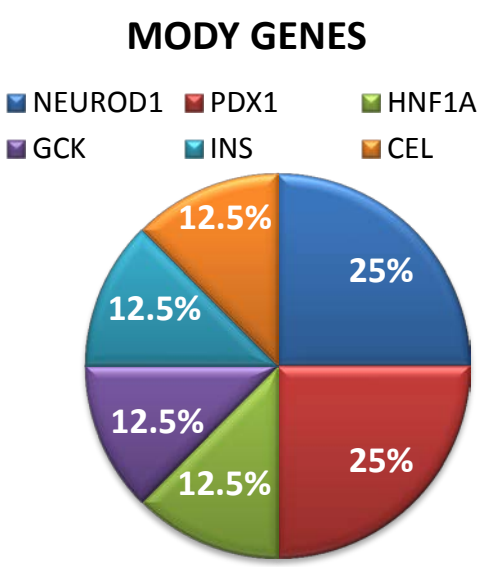
In our study, seven (26%) of the 27 pregnant women who had a 3 generation family history and autosomal dominant form of diabetes were positive for MODY mutation, whereas only one (4%) of the 23 subjects without 3 generation family history of diabetes was positive for MODY mutation. The MODY positivity was significantly higher in those with a 3 generation family history of diabetes with an odds ratio (OR) of 1.291[CI=1.016-1.641; (p= 0.038)]. This is similar to earlier reports where selective screening for mutations in subjects with the typical phenotype with presence of family history of diabetes has shown prevalence ranging from 12-80%. The higher frequency of mutations found in our study may be attributed to the presence of significant number of subjects with pregestational young onset diabetes and more than 50% of the subjects having an autosomal dominant inheritance of diabetes, which are hallmarks of MODY. The median age at diagnosis of MODY positive subjects in our study was 29.6 (range 10-36 years). Fajan and Bell in a review on MODY have highlighted that, though young onset of diabetes is important, the initial criteria specifying the age at diagnosis to be less than 25 years should no longer be essential (79).

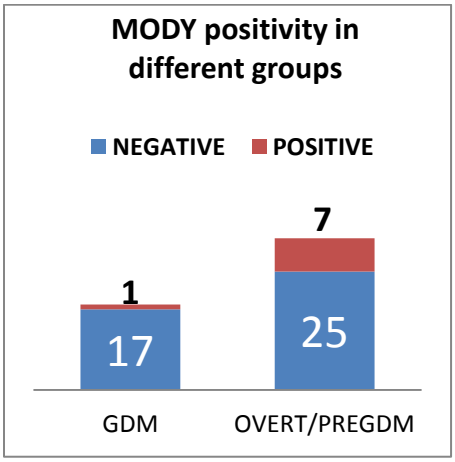
Subjects with overt diabetes and pregestational diabetes had a significantly higher prevalence of mutation (7/32) in comparison to women with gestational diabetes(1/18, p=0.017). Though, overall there was no significant difference among the subjects with respect to the age at diagnosis, BMI and birth weight of offspring, subjects with NEUROD1 mutation had a higher mean BMI (31.1kg/m^2) than those with other mutations (27kg/m^2).

Interestingly in this study population, utilising the NGS platform we have detected a significantly higher frequency of mutations in NEUROD1 & PDX1 genes {25% (n=2) each in MODY positive subjects} in comparison to the more commonly reported GCK, HNF1A gene mutations {12.5% (n=1) each in MODY positive subjects}. The other two mutations reported were in CEL and INS genes. This is similar to another study done at our centre[#], where we found a higher frequency of NEUROD1 mutations in subjects of Asian-India origin with autosomal dominant young onset diabetes. In the literature reported on MODY mutations in GDM, GCK mutations have been the commonest followed by HNF1A, HNF4A, PDX1 and INS genes. Till date, there are no reported NEUROD1, CEL, PAX or KLF mutations in women with gestational diabetes. This may be attributable either to the low prevalence of mutations in the screened population or due to the sequential screening by the conventional Sanger sequencing methodology, which is limited by cost and scalability. The higher prevalence of MODY mutation in our study may be attributed to the parallelized sequencing of a panel of 10 genes and partly to the inclusion of young women with pregestational diabetes. The higher frequency of NEUROD1 and PDX1 mutations in our population may be due to differential prevalence of the various MODY mutations across the globe (racial and ethnic differences).

[#] The study was presented at the 63rd annual American society of human genetics conference. 2013. Boston, USA http://www.ashg.org/2013meeting/abstracts/fulltext/f130122650.htm







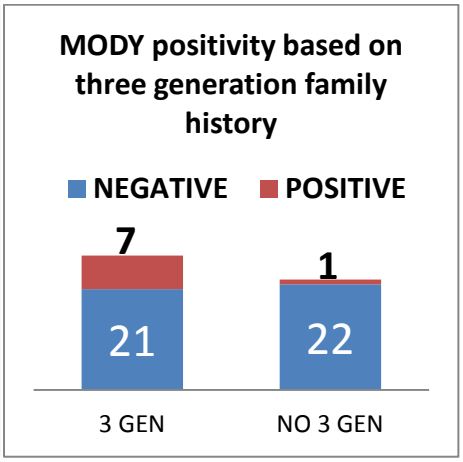


Table: 12A: PREDICTION OF PATHOGENICITY BY THE BIOINFORMATICS TOOLS

MEPN	MODY	GENE	Mutation	Aminoacid change	SIFT	Polyphen-2	MUTATION TASTER
MG95	**MODY 2**	**GCK**	**c.1042T>A**	Ile348Phe	Damaging	Probably damaging	Disease causing
MG53	**MODY 3**	**HNF1A**	**c.8C>G**	Ser3Cys	Damaging	Possibly damaging	Disease causing
MG101	**MODY 4**	**PDX**	**c.670 G>A**	Glu224Lys	Damaging	Possibly damaging	Disease causing
MG19	**MODY 4**	PDX	**c.282A>G**	His94Gln	Tolerated	Benign	Disease causing
MG86	**MODY 6**	**NEUROD1**	**c.175 G>C**	Glu59Gln	Tolerated	Benign	Disease causing
MG28	**MODY 6**	NEUROD1	**c.953A>G**	Phe318Ser	Damaging	Benign	Disease causing
MG102	**MODY 8***	**CEL**	**c.248T>C**	Phe83Ser	Tolerated	Probably damaging	Polymorphism
MG42	**MODY 10**	INS	**c.130C>T**	Gly44Arg	Damaging	Probably damaging	Disease causing

***SIFT-http://sift.bii.a-star.edu.sg/**

****Polyphen-2 http://genetics.bwh.harvard.edu/pph2/**

*****MutationTaster-http://www.mutationtaster.org/**

GENOTYPE – PHENOTYPE CORRELATION (GPC):

The mutational, clinical details and pedigree chart of all the MODY positive subjects are summarised in detail earlier in results {Table-12, 12A, GPC: I-VIII}. All the variants were predicted to be disease causing by Mutation-Taster except F318S CEL variant which was predicted to be probably damaging by polyphen-2. ***SIFT** predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids. SIFT can be applied to naturally occurring nonsynonymous polymorphisms and laboratory-induced missense mutations(148). ****PolyPhen**-2 is a tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations(147). Unlike SIFT or PolyPhen which handle only single amino acid substitutions, ***** **MutationTaster**(149) works on DNA level for prediction and allows insertions and deletions.

1. MODY 2: GLUCOKINASE(GCK)(GPC-I)

Based on earlier reports the most common reported MODY gene mutation is GCK with prevalence between 0-80%, which depends on the selection criteria utilised for selecting subjects for genetic screening. In our study, one subject (2% overall and 4% in subject (MG95) with 3 generation family history of diabetes) was detected to have GCK mutation (novel p.I348F) which is similar (2.5 – 5%) to that, reported by Stoffel et al (1993), Weng et al (2002) and Zurawek et al (2007) (Table-4). She (M95) was detected to have overt diabetes when screened at 21 weeks of gestation with HbA1c of 7%. She had inherited the gene from her father who was diagnosed to have diabetes at the age of 45 years and he is currently being managed with SU therapy. In a detailed review, Fajans et al. (2001) stated that glucokinase-related MODY2 is a common form especially in children with mild hyperglycemia and in women with gestational diabetes(154). About 50% of the women who are carriers may have gestational diabetes. It has been

described in persons of all racial and ethnic groups. More than 130 MODY-associated mutations have been found in the glucokinase gene. The mild fasting hyperglycemia with blood glucose concentrations of 110 to 145 mg/dl and impaired glucose tolerance in most affected carriers may be recognized by biochemical testing at a young age, possibly as early as birth. Heterozygous mutations in glucokinase are associated with a mild form of non progressive hyperglycemia that is usually asymptomatic and can be managed with diet alone. Less than 50% of the carriers have overt diabetes; many of those who do, are obese or elderly and only 2% percent of MODY2 patients require insulin therapy. Some studies have noted that as GCK acts as the beta cell glucose sensor, fetuses that carry a GCK mutation are not able to mount an appropriate insulin response to the prevailing glucose levels and do not display the fetal growth that would be expected from maternal glycemia(98).

Our subject was managed with Insulin for glycemic control. Postpartum her blood glucose levels have normalised. She had GDM in her previous pregnancy and was managed with Insulin. During pregnancy it is very likely that women with DM due to a GCK mutation will present with raised blood glucose values usually prompting insulin therapy. Glyburide (glibenclamide) has not been tested in pregnant women with a GCK mutation but the fact that sulfonylureas increase GCK expression in vitro(7) would open the possibility that these women may be treated with glyburide. In addition, in view of the opposing effects of maternal hyperglycemia and the presence of a fetal GCK mutation (101,108–112), maternal treatment should take into account the fetal genotype. Barrio et al. (2002) have noted that the birth weight was lower in the presence of a GCK fetal mutation when the mutation was of paternal origin(109). The problem is that the fetal genotype is not known and it does not seem justifiable to use a chorionic biopsy/ amniocentesis for this purpose. The current genetic status of the children of our subject is yet to be determined, but the birth weight of both the neonates was normal. Though

currently she has normal blood glucose levels, she needs to be closely followed up for the development of either isolated fasting hyperglycemia or diabetes. As seen in our study, Cuesta-Muñoz et al(155) have clearly demonstrated the heterogeneity in the clinical expression within a family with glucokinase mature-onset diabetes of the young (GCK-MODY).

2. MODY3 - HEPATOCYTE NUCLEAR FACTOR-1 ALPHA.(GPC-II)

Mutations in the HNF1A gene encoding hepatocyte nuclear factor-1 alpha have been previously delineated as the most common cause of MODY in most adult populations and reported to have a prevalence that ranges between 11 to 50% (154)(156)(157). However, in the present study we have found only one subject viz., MG53 with this mutation which bears similarity to earlier reports which have detected HNF1A mutations in 1% subjects with GDM. She was detected to have a novel S3C HNF1A mutation. The number of different pathogenic HNF1A mutations totals 414 in 1,247 families (158). She was diagnosed to have diabetes at the age of 22 years. In her first pregnancy, she had poor glycemic control in the first trimester and was managed with Insulin during the pregnancy. She delivered a male neonate with congenital heart disease, transposition of great vessels (TGV) with pulmonary atresia and the baby expired at 1 month. Subsequently with an effort to achieve good glycemic control she was started on Insulin and conceived for the second time with good glycemic control during conception. She delivered a healthy female baby with macrosomia (3940grams). She is currently on Insulin and SU therapy for glycemic control as she is planning for third pregnancy. Sensitivity to therapy with sulfonylurea is a feature of both HNF1A and HNF4A mutations. The hyperglycemia in patients with MODY3 tends to increase over time, resulting in the need for insulin in many of these patients (30 to 40% require insulin) (154). Patients with MODY3 may have the full spectrum of complications of diabetes (more than 40% of all relatives of MODY3 patients) and are as common in

these patients as in patients with type I or type 2 diabetes (matched according to the duration of diabetes and the degree of glycemic control) and are probably determined by the degree of glycemic control (109). MG53 has inherited the mutation from her father and her child is also a carrier of the same mutation. Her father who was diagnosed to have diabetes at the age of 32 years is currently managed with SU therapy. Klupa et al have described that in comparison to carriers of mutations of paternal origin, those with a mutation of maternal origin displayed a shift to the left in the curves of cumulative risk of DM and the requirement for insulin (116). Our subject, currently requiring insulin to maintain good glycemic control, after completion of family needs to be evaluated for response to SU therapy and her child should to be carefully followed up.

2. MODY 4 - PDX1 (PANCREATIC AND DUODENAL HOMEOBOX 1) (GPC-III&IV)

PDX1 (known as IPF1: Insulin Promoter Factor 1) mutations that are a rare cause of MODY with pancreatic agenesis, have increasingly been shown to predispose to apparently monogenic early onset type 2 diabetes mellitus, with the phenotype depending upon severity of the mutation (159)(160)(161). In the present study we identified PDX1 mutation in two subjects MG19 with overt diabetes and MG101 with pre-gestational diabetes. M19 has a novel H94Q PDX1 mutation. She was a multigravida with two previous preterm caesarean deliveries and low birth weight. Her current child was preterm with birth weight of 3300 grams. Post-delivery she has diabetes and is currently managed with metformin and SU. Her family members and neonate are yet to consent for mutation screening. MG101 had pregestational diabetes, who was diagnosed to have diabetes at the age of 10 years with ketoacidosis at onset and has been on Insulin since then. She was negative for autoantibodies and hence was screened for MODY mutation. She was detected to have a reported E224K PDX1 mutation (162). She also delivered a preterm baby with low birth weight (2240 grams) who is yet to be screened. The glycemic status of mother was not available. The E224K

mutation was found in two unrelated diabetic Indo-Trinidadians (162) and cosegregated with early-onset diabetes or impaired glucose tolerance in family members, suggestive of the type 4 form of maturity-onset diabetes of the young. Functional studies of E224K showed reduced transactivation activity, leading to synthesis of a mutant protein and hence reduced Insulin secretion(162). There are also reported cases of A−β−Ketosis-prone diabetes (KPD) with variants in HNF1A, PDX1 and PAX4 genes (163)(164). Gragnoli C et al in their study found that 4 out of the 5 female pregnant women who were carriers of PDX1 mutation, had their pregnancies complicated by reduced birth weights, miscarriages, or early postnatal deaths, similar to that noticed in our subjects(121). The IPF1 mutant protein (P33T) showed a reduced DNA-binding and transcriptional activation function as compared to the wild-type PDX1 protein in studies in vitro (121). These findings suggest that the earlier reported P33Tand E224K PDX1 mutation along with the H94Q PDX1 mutation may provide an increased susceptibility to the occurrence of MODY4andgestational diabetes, low birth weight in their offspring.

4. MODY 6 : NEURO D1 NEUROGENIC DIFFERENTIATION FACTOR 1 (GPC-V & VI)

NEUROD1 mutations (MODY 6) have been reported from only six families(122–125). But in our population, two subjects MG86and MG28 were found to have novel E59Qand F318S NEUROD1 mutations respectively in a highly conserved region with a GERP score of 5.9. MG86 was diagnosed to have overt diabetes and she delivered a macrosomic male baby (3700grams) by caesarean section. Postpartum at 12 weeks she was detected to have diabetes and is currently on metformin with glipizide. She has inherited this mutation from her mother who was diagnosed to have diabetes at the age of 40 years. The neonate is a carrier of this mutation. Her mother was also obese and interestingly had delivered a macrosomic baby with birth weight of 4000 grams in 1974. Her sister had gestational diabetes and is yet to be screened for mutations.

MG28 with F318S NEUROD1 mutation was diagnosed to have pregestational diabetes at the age of 27 years. She is obese with BMI of 30kg/m2. She had delivered a preterm low birth weight male baby (2020 grams) by caesarean section and in the current pregnancy she gave birth to a female term baby with normal birth weight. She has inherited the mutation from her father and her first child with low birth weight is a carrier.

In contrast to our study, in a Norwegian study (94) on MODY X patients no NEUROD1 mutations were found and hence had previously suggested that NEUROD1 may not be a candidate gene in gestational diabetes mellitus (GDM) in that particular population. In Malecki et al. (1999) described 2 heterozygous mutations in NEUROD1 which were associated with the development of type 2 diabetes. One was a missense mutation arg111-to-leu that disrupts the DNA-binding domain and abolishes the E-box binding activity of NEUROD1. The second mutation, 206+C gives rise to a truncated polypeptide lacking the C-terminal transactivation domain, a region that associates with the coactivator p300. The clinical profile of patients with the truncated NEUROD1 polypeptide was more severe and required insulin for treatment, whereas that of patients with the arg111-to-leu mutation was more typical of type 2 diabetes mellitus. NEUROD1 His241Gln mutations have been reported in families with obesity (125). Although the direct pathogenic association with the clinical phenotype of some NEUROD1 variants remains unclear (122–125), we hypothesize that the additional diabetogenic factor conferred by increased body weight seen in our subjects with NeuroD1 mutations (mean BMI: 31kg/m2) may have contributed to the translation of beta-cell dysfunction into diabetes at an early age. A similar phenomenon of increased body weight required for translation of β-cell abnormalities into diabetes has been previously reported with B lymphocyte tyrosine kinase (BLK MODY 11) gene mutations (165).

5. MODY 8: CEL:CARBOXYL ESTER LIPASE MUTATION(GPC-VII)

MODY8 can be caused by frameshift deletions in the variable number of tandem repeats (VNTR) of the carboxyl-ester lipase gene (CEL).The pancreas serves both endocrine and exocrine functions. The localization of the islets within exocrine pancreatic tissue is suggestive of an interdependency and crosstalk between these two cell populations in their normal and in their abnormal function (127)(166). In our subject MG102 we identified a novel F318S CEL mutation. She was diagnosed to have gestational diabetes. She has two children delivered by caesarean section. Both were of normal birth weight. She has inherited this mutation from her mother who was diagnosed to have diabetes at the age of 42 years and is on SU therapy. Her sister was also diagnosed to have GDM at the age of 24 years; she has two children and her second child was delivered preterm with low birth weight. At present her glycemic status is not yet determined as she is yet to come back for screening by OGTT. Her children also are yet to be screened. Till date no CEL mutations have been reported with GDM.

Using the multiplex PCR method, Torsvik et al(167) screened 56 members of the two Norwegian families previously studied by Raeder et al(127) in 2006 and confirmed the presence of single-base deletions in the 23 affected individuals. In the same study Torsvik had also analyzed the CEL VNTR in 95 Danish probands who were negative for mutations in the first 7 known MODY genes and found one proband with a very short VNTR allele, consisting of only 3 repeats. Of the seven 3-repeat VNTR carriers in the proband's family, 4 had diabetes, 1 had impaired fasting glucose(IFG), and 1 had impaired glucose tolerance(IGT); family members with normal VNTR lengths were all normal glucose levels(167). Further, there was no difference in BMI between carriers and non-carriers in the family. In the Danish family there was no evidence of cosegregation with exocrine deficiency(167) and the 3-repeat VNTR allele appeared to have lower penetrance for diabetes than the mutations previously described in the

Norwegian families(127). Similarly our subject (MG102) and her family members who were screened there was no evidence of exocrine pancreatic insufficiency.

6. MODY 10 : INS: INSULIN GENE MUTATION(GPC-VIII)

Insulin gene (INS) mutations (MODY 10) are a common cause of permanent neonatal diabetes (PNDM) and a very rare cause of diabetes diagnosed in childhood or adulthood. In the present study, MG42 was detected to have a novel G44R INS mutation. She was diagnosed to have overt diabetes in her second pregnancy. She has two children with normal birth weight born at term. In a study of maturity-onset diabetes of the young (MODY-X) patients by Boesgaard et al, a novel heterozygous c.17G>A, R6H mutation was identified in the pre-proinsulin gene (INS) in a Danish MODYX family with one 27 year old GDM(126). Approximately 30% reduction in beta-cell function measured as insulinogenic index was noted in this family. In the same study a previously described R46Q mutation was found in a Czech MODYX family and they were treated with oral hypoglycemic agents and/or insulin(126). Edghill et al(168) has also described a family with MODY who carried a heterozygous missense R6C mutation in the INS gene. Molven et al(169) described a proband with R46Q INS mutation who was diagnosed with diabetes at 20 years of age. Two of his relatives were managed with diet alone for more than 10 years, the family members with diabetes were non obese and all except one did not require Insulin. Postpartum, our subject MG42 has IGT and is managed with diet modification alone. Both her parents, her grandfather and paternal uncle who had diabetes have expired. Her children are not carriers of this mutation.

COST-EFFECTIVENESS OF NGS:

At present, Sanger sequencing of three MODY genes [HNF1A, GCK and HNF4A] costs around $2000, with a turnaround time of 40 days for reporting a mutation (170). Utilizing the (2GDMODY protocol) Ion Torrent PGM, genetic screening of a panel of ten MODY genes, with a flexibility of sequencing 3 to 10 samples utilizing Ion 314 or 316 chips, it would cost less than $500 with a turnaround time of 30days. Furthermore, the specific variants identified by NGS can be confirmed by Sanger sequencing. Therefore, these NGS technologies that deliver fast, inexpensive, and accurate genomic information, will be a useful tool in the diagnosis and management of heterogeneous genetic disorders like MODY.

SECTION-II:

Genome wide association studies in T2DM have shown that *TCF7L2* variants represent one of the strongest genetic risk alleles associated with the occurrence of diabetes. Furthermore, TCF7L2 polymorphism have been studied in various populations and found to have significant association with increased risk of gestational diabetes (171)(172)(173)(174). These genetic studies have shown that the *TCF7L2* rs7903146 variant is associated with an increased risk of gestational diabetes mellitus in Scandinavian women (OR= 1.49 [95% CI 1.28–1.75], $p = 0.049$ (171) and in European populations of primary caucasian origin (p=0.011; (OR)=3.17; 95%CI=1.318-7.665) (172). TCF7L2 rs12255372 variant is associated with an increased risk of gestational diabetes mellitus in Arab women (OR=2.370, (95% of CI 1.010–5.563) with the p = 0.047) (173), and in Caucasian women (OR=7.7, 95% CI: 1.71–34.60) (174). However there is no reported study that has looked at the role of TCF7L2 variants and their association with gestational diabetes in Indian population. In our study, we assessed the

associations of three *TCF7L2 single nucleotide polymorphisms* (SNP) (rs7903146, rs12255372 and rs4506565) with gestational diabetes in 117 pregnant women. Though the TCF7L2 variant rs4506565 has not been studied in patients with gestational diabetes it has been shown to be associated with type 2 diabetes mellitus in Indian population (OR=1.48 [95% CI=1.24–1.77], p=2.0×10−5) (29). We have found that the odds ratio (OR) of TCF7L2 polymorphisms rs4506565, rs7903146, rs12255372 for the occurrence of gestational diabetes when compared with controls were 3.75(C.I=0.75-18.53, P=0.08), 1.77 (C.I=0.503-6.263, P=0.37) and 1.40 (C.I=0.24-8.11, P=0.70) respectively.

Our results were closest to studies on Arab population by *Nasser Rizk et al* (173)and in European population *by Potasso et al*.(172). The TCF7L2 polymorphism rs4506565 has showed a strong trend towards association with gestational diabetes, when compared to the other two common polymorphisms in TCF7L2 (rs7903146, rs12255372).

This is the first preliminary data of TCF7L2 polymorphism associations with gestational diabetes in a south Indian population. However, this study needs to be further performed in a large population to confirm our results and establish the risk of TCF7L2 polymorphism in the occurrence of gestational diabetes.

1. In the present study, 50 women of Asian Indian origin with diabetes complicating their pregnancy were screened for a comprehensive panel of ten MODY genes utilizing a novel multiplex PCR-based next generation sequencing on the Ion torrent PGM.
2. We have identified MODY mutations in 16% (8/50) of the subjects.
3. A higher frequency of NEUROD1 and PDX1 (two each) mutations were identified. The other mutations detected were one each in HNF1A, GCK, CEL and INS genes. Seven of these mutations were novel. One subject MG101 had a reported E224K PDX1 mutation.
4. Seven (26%) of the 27 subjects with a 3 generation family history and autosomal dominant form of diabetes were positive for MODY mutation, whereas only one (4%) of the 23 subjects without a 3 generation family history of diabetes was positive for MODY mutation. The MODY positivity was significantly high in those with 3 generation family history diabetes with odds ratio of 1.291[CI=1.016-1.641; (p= 0.038)].
5. Mutations were identified in four pregnant women with overt diabetes (25%), three with pregestational diabetes (20%) and one with gestational diabetes (4%).There was significantly increased predisposition to MODY mutation positivity in pregnant women with overt and pregestational diabetes group in comparison to those with GDM (p=0.017).
6. Although there appeared to be no significant difference among the subjects with regard to the age at diagnosis, BMI and birth weight of offspring among those with MODY mutations, subjects with NEUROD1 mutation had a higher BMI (31.1kg/m^2) than those with other mutations (27kg//m^2).
7. All except two subjects (MG101 with the reported E224K PDX1 mutation and MG53 with S3C HNF1A mutation) are currently managed with dietary modification, metformin or sulphonylurea (SU) therapy.
8. Further in subjects with GDM, the odds ratios (OR) of association of TCF7L2 polymorphisms rs4506565, rs7903146, rs12255372 with gestational diabetes when compared to controls were 3.75(C.I=0.75-18.53, P=0.08), 1.77(C.I=0.503-6.263, P=0.37) and 1.40(C.I=0.24-8.11, P=0.70) respectively.

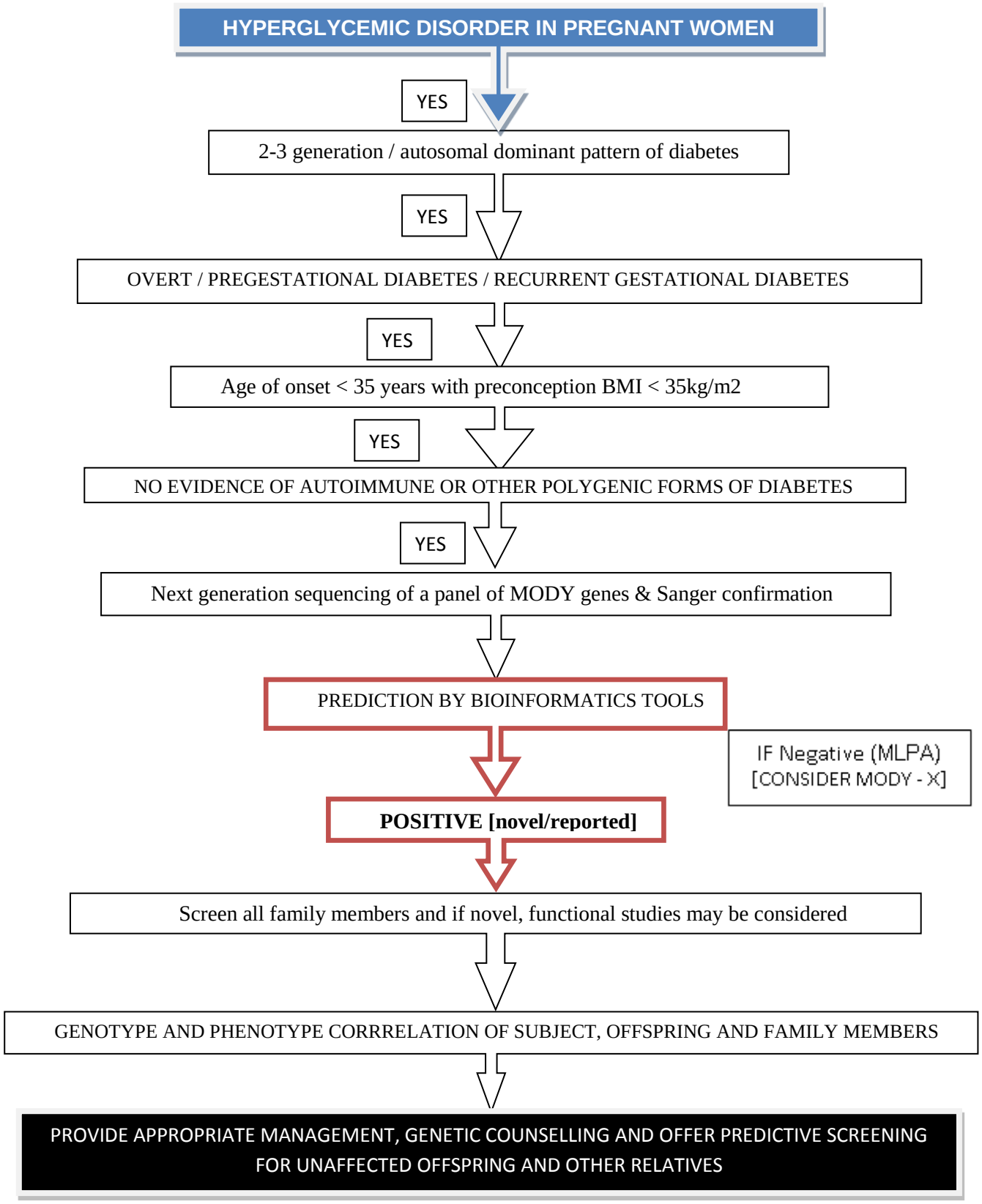
1. Sixteen percent (8/50) of pregnant women of south-Indian origin (Asian Indian) harboured mutation in one of the 10 MODY genes. Among them, women with overt and pregestational diabetes (20%) showed a significantly increased predisposition towards the detection of a MODY mutation in comparison to those with gestational diabetes (4%). Further, MODY positivity was significantly high in pregnant women with a 3 generation family history of diabetes.
2. NEUROD1 & PDX1 mutations are more frequently detected in our population than the commonly reported GCK and HNF1A mutations by western literature in Caucasian population.
3. Next Generation Sequencing on Ion Torrent PGM provided a cost-effective, rapid, accurate method to identify mutations in a comprehensive panel of MODY genes overcoming the limitations of conventional sequential screening by Sanger's methodology. With the recent FDA approval of one of the Next Generation Sequencing platforms, once validated for its specificity and sensitivity, this technology would aid the medical fraternity for massive parallel sequencing of target genes that are of diagnostic and therapeutic significance in large families with undiagnosed MODY, even in developing countries like India.
4. The methodology used over here for MODY genetic screening is useful for screening siblings and the new born children at an early point in life with regards to the presence of a mutation, and would be invaluable in instituting early preventive measures in them and their family members.
5. The TCF7L2 polymorphism rs4506565 has showed a strong trend towards an association with the occurrence of GDM, when compared to the other two common polymorphisms (rs7903146, rs12255372).

1. The subjects screened are not representative of the entire population of gestational diabetes owing to a referral bias of those women who were not controlled with diet alone, and required addition of oral glucose lowering agents/ insulin for optimal glycemic control. It is likely that a slightly different pattern may be obtained in those with mild hyperglycemia requiring diet modification alone. However women with hyperglycemic disorders diagnosed early in pregnancy should be considered for screening for MODY mutations especially if they have an autosomal dominant inheritance pattern of diabetes in the family members.
2. The novel variants identified in this study were absent in 50 control subjects and the 1000 genome database were predicted to be causative by at least one of the bioinformatics tool used in this study. However, further functional and family studies are required to investigate and confirm the role of these novel variants in the pathogenesis of MODY.
3. Currently, due to a high rate of indels (insertions and deletions) in homopolymer stretches (175,176), Ion Torrent PGM could miss true indels and therefore require multiplex ligation-dependent probe amplification (MLPA) (177) assay to detect deletions or duplications. However, with recent improvements in the sequencing chemistry, it is expected to significantly reduce indel errors at the homopolymer stretches (178).
4. A potential limitation of this study on TCF7L2 polymorphism is the relatively small number of patients and control subjects; thus, estimates of the association have relatively large confidence intervals, especially for homozygous subjects.

In contrast to earlier reports, our study has not only detected a different pattern of genetic mutations with variable phenotypes in pregnant women of Asian Indian origin, but also it has highlighted that Next-generation sequencing (NGS) provides a cost-effective, rapid, accurate method to identify mutations in a comprehensive panel of MODY genes. The important implications and future prospects of our study are:

1. Targeted parallel screening for MODY mutation in women with overt diabetes with a significant family history would help in identifying more women at early risk of diabetes. These women can be counselled for frequent screening for glucose intolerance and provide early intervention depending on the type of mutation. These women can receive appropriate pre-conceptional counselling and achieve adequate glycemic control prior to subsequent conception.
2. The mutation positive women who achieve normoglycemia in the post-partum period are at a very high risk in developing overt/ gestational diabetes in future pregnancies and hence early screening and intervention may reduce maternal and fetal complications related to hyperglycemia.
3. The screening of first degree relatives and other family members of women with pregestational and/or overt diabetes along with genotype – phenotype correlations will provide an opportunity to understand the heterogeneity, penetrance and pathogenicity of a MODY mutation. This will also help to decipher the role of specific mutations in the occurrence of macrosomia, preterm deliveries, low birth weight, pregnancy loss, neonatal complications, diabetes complications and response to therapy.
4. MODY, being an autosomal dominant disorder provides the opportunity to screen offspring for the presence of specific mutation. Subsequently, they could be counselled for early detection of glucose intolerance and provide interventions for delaying the onset of diabetes.
5. The presence of known mutations will also help in appropriate streamlining of therapy and screen for complications and co-morbidities. {eg. Subjects with GCK usually are managed with diet modification, are less likely to develop complications; where as those with HNF1A mutation responded to SU therapy, may require Insulin and are equally prone to complications.}
6. Screening of large populations may be done to study the role of other rare variants / polymorphisms in the pathogenesis of MODY/GDM.

SUGGESTED ALGORITHM FOR SCREENING OF MUTATIONS IN MODY GENES IN PREGNANT WOMEN WITH DIABETES



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Department of Endocrinology, Diabetes and Metabolism
Christian Medical College & Hospital, Vellore.

INFORMED ADULT CONSENT FORM

STUDY TITLE

The Study of Pregnant Women with Diabetes and Screening for Mutations Related to MODY (Maturity Onset Diabetes of the Young) genes and polymorphisms in TCF7L2.

PRINCIPAL INVESTIGATOR
Dr. Nihal J. Thomas

A. I _____, understand that the physicians at the department of Endocrinology, Christian Medical College are engaged in research on the nature, diagnosis and treatment of Diabetes.

B. I have been informed that this study involves research and that the purpose is to assess the genetic basis of monogenic forms of diabetes and the following genes would be sequenced to find the genetic variants.

HNF1A, HNF4A, GCK, HNF1β, IPF1, NEUROD1, KLF11, CEL, PAX4 & INS MODY GENES AND TCF7L2 POLYMORPHISM

C. I understand that a sample of blood (10ml) will be drawn once for research purposes and not as a routine clinical test, and that this blood sample (or the material in it, including genetic material) may be stored and used in the laboratory for many years, in relation to treatment objectives of this study until it is used up.

D. I understand that the possible discomforts and risks attendant to this procedure include some degree of pain from the insertion of the needle into the vein, but this is usually minimal. An occasional patient faints, and rarely there is some discomfort for a few days at the site of venipuncture due to the formation of a bruise. If any tests are performed on my blood, I will be told which tests have been performed and the results could be made available to me.

E. I understand that the records connected with my participation in the clinical investigation will be kept strictly confidential. My name will not be revealed in any publication that may arise from this study.

F. I have had the opportunity to ask questions concerning the procedures to be used. I understand that if I have any further questions concerning the research conducted, I may contact Dr. Nihal Thomas or his colleagues in the department of Endocrinology, Christian Medical College, and Vellore.

G. I further understand that I am free to withdraw my consent at any time without any penalty or loss of benefits to which I may be otherwise entitled. I also will not be paid for participation in this study but there will be no additional cost to me for evaluations necessary for this study

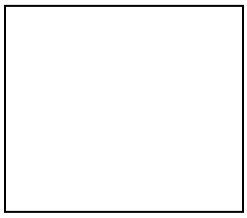
H. I also give permission for the doctors to collect 1ml of blood from my baby after birth, if the doctors advise genetic testing. I understand that blood collection on neonates is a routine procedure, and does not have any adverse effects on the health of my baby.

I. I hereby voluntarily consent to participate in this study and to allow the procedures described above to be performed on me.

_____ _____

Signature of Subject Date

OR



Thumb Impression

INVESTIGATOR'S OR SUB-INVESTIGATORS STATEMENT

I have offered an opportunity for further explanation of this procedure to the individual whose signature appears above.

_____

Note: The subject shall be given a copy of this consent form. A signed copy must be filled in the patient's file at the Department of Endocrinology, Christian Medical College & Hospital, Vellore.

CONSENT FORM FOR NEWBORN INFANT

Title of the Project: The Study of Pregnant Women with Diabetes and Screening for Mutations Related to MODY (Maturity Onset Diabetes of the Young) genes and polymorphisms in TCF7L2.

Principal Investigator: Dr. Nihal Thomas

I have read the Patient Information Sheet and have been explained the nature of the study and I am aware of the following facts:

1. I have been invited to allow my child to participate in a research project as mentioned above, because my baby meets the eligibility criteria for the study.

2. If I give consent, a blood sample (1mL) would be collected to test and see whether the genetic variations that are present in me are present in my child as well.

3. I will not be provided monetary compensation for the expenses incurred.

4. No financial compensation will be provided for any study-related adverse outcome.

5. I have the option of not giving consent for participation or of withdrawing from the study at any point of time. If I do exercise these options, my child will continue to receive treatment as per current standards.

6. All information gathered in the course of the study will be kept confidential.

7. I have been provided the contact information of the investigators, as above.

8. The above information is in a language that I can understand.

9. I understand that the study staff and institutional ethics committee members will not need my permission to look at my baby's health records even if I withdraw from the trial.

10. I understand that my baby's identity will not be revealed in any information released to third parties or published.

I hereby give my voluntary consent to enroll my child, baby of _____ in the above study.

Signature / Thumb impression:
Name:
Relationship with the child:
Date:

Signature of Investigator:
Name:
Date:

Witness signature:
Witness name:

PARTICIPANT INFORMATION SHEET

You are being invited to participate in a research study. Your participation in this study is entirely voluntary. Your decision whether or not to participate will have no effect on the quality of medical care you will receive in this hospital.

An investigator in this study will explain the purpose of this study including how this study will be carried out and your contribution or role in the study. The paragraphs below explain the study. Please ask questions if there is anything you do not understand before you decide to participate in this study.

STUDY TITLE

Gestational Diabetes Mellitus – The Window of Opportunity to Prevent Diabetes in two Generations

PRINCIPAL INVESTIGATOR: Dr. Nihal Thomas

What is the purpose of this study?
This study plans to look at why you, as a young pregnant woman, have increased tendency to develop diabetes. Genetic disorders form a small proportion of the patients who have diabetes. However, it is not known in India as to what the common genetic causes of diabetes and glucose intolerance in pregnancy, and how common they are. This study aims to determine whether there is a genetic predisposition to causediabetes during pregnancy,at an early age.

The genes that we will try to determine include:

HNF1A, HNF4A, GCK, HNF1β, IPF1, NEUROD1, KLF11, CEL, PAX4 & INS MODY GENES AND TCF7L2 POLYMORPHISM

What does this study involve?
The blood samples obtained from you will be tested for genetic variations. If a positive genetic variation is found in your sample, we will collect 1ml of blood from your baby, after birth, to study for the same genetic variation.

Are there any benefits from participating in this study?
These tests will provide a genetic confirmation of your diagnosis. If there is a genetic mutation, based on the type, changes in treatment will be made, and family members can be counseled based on this. On the other hand, if no genetic mutations are detected, no useful information can be provided.It may however, help to increase your awareness about diabetes. This study is helpful for science and humanity.It is possible that patients, especially young pregnant women who have diabetes, later may benefit.

What are the risks involved in participating in this study?
Participating in this study does not affect your pregnancy or the health of your baby in any adverse manner. You will receive the same standard care regardless of your willingness to participate in the study.

Will my taking part in this study be kept confidential?
All information collected about you during the course of research will be kept strictly confidential. Any information which leaves the hospital/clinic/laboratory will have your name and address removed so that you cannot be recognized from it.

Information on stored specimen?
The blood samples obtained from you will be stored at -70dec C at the Department of Endocrinology, Diabetes, & Metabolism. It will be used for extraction of DNA to study the genetic mutations.

What will happen to the results of the research study?
Results of the research will be published in national/international journals. We are also assuring you that you will not be identified in any report/publication.

If the above information does not provide answers to your questions, request the investigator to answer them for you. Also, this sheet is for your information and you can retain this copy with you.

Contact for further information-
For further information kindly contact-

Dr Nihal Thomas, MD, DNB (Endo), MNAMS (Endo), FRACP (Endo), FRCP (Edin)
Professor & Head
Department of Endocrinology, Diabetes and Metabolism,
Christian Medical College and Hospital, Vellore -632004, Tamil Nadu, India.
Office Ph no: 0416-2282694, 0416-4200844

Thank you for taking part in this research study.

Contact for further information-

For further information kindly contact-

Dr Nihal Thomas, MD, DNB (Endo), MNAMS (Endo), FRACP (Endo), FRCP (Edin)
Professor & Head
Department of Endocrinology, Diabetes and Metabolism,
Christian Medical College and Hospital, Vellore -632004, Tamil Nadu, India.
Office Ph no: 0416-2282694, 0416-4200844

Thank you for taking part in this research study.

அகச்சுரப்பியல், நீரிழிவு நோய் மற்றும் மெட்டபாலிசம் துறை

கிருத்துவ மருத்துவக் கல்லூரி, வேலூர்

கர்பக்காலத்தில தோன்றும் நீரிழிவு நோய் குறித்த மரபணு

ஆராய்ச்சி தலைமை ஆராயச்சியாளர்: டாக்டர் நிஹால் தாமஸ்

தகவல் அறிதல் மற்றும் ஒப்புதல படிவம்

..... ஆகிய நான், சி.எம்.சி. அகச்சுரப்பியல் மற்றும் நீரிழிவு நோய் துறையின் மருத்துவர்கள் சர்க்கரை நோயின் தன்மை நோயறிகை மற்றும் சிகிச்சை முறை பற்றிய ஆராய்ச்சியில் ஈடுபட்டிருக்கிறார்கள் என்பதை புரிந்து கொள்கிறேன்.

கர்பக்காலத்தில் தோன்றும் நீரிழிவு நோய்க்கு மரபு வழிக்காரணி உள்ளதா என்பது பற்றிய இந்த ஆய்வில்

கிழ்க்கண்ட மரபணுக்கள் பரிசோதிக்கப்படும் என்பது எனக்குத் தெரிவிக்கப்பட்டது.

HNF1A, HNF4A, GCK, HNF1β, IPF1, NEUROD1, KLF11, CEL, PAX4 & INS MODY GENES AND TCF7L2 POLYMORPHISM

இந்த ஆராய்ச்சிக்காக என்னிடம் 10 மிலி இரத்தம் ஒரு முறை மட்டும் எடுக்கப்படும் என்றும், அந்த இரத்தம் பல ஆண்டுகள் வரையில் பதப்படுத்தி வைக்கப்பட்டு உபயோகப்படுத்தலாம் என்பதை நான் அறிவேன்.

இரத்தம் எடுக்க ஊசியை குத்தும் போது சற்று வலிக்கும் என்பதை நான் அறிவேன். அவ்வாறு இரத்தம் எடுக்கும் போது அரிதாக ஒரு சிலருக்கு மயக்கம்

மற்றும் ஒரு சிலருக்கு அவ்விடத்தில் சிராய்ப்பினால் அசௌகரியமோ ஏற்படலாம். இந்த இரத்த சோதனை நடத்தியப்பிறகு, அதன் முடிவுகள் எனக்கு தெரிவிக்கப்படும்.

இந்த ஆராயச்சியினால் வளரும் என் குழந்தைக்கு எந்த விதமான பக்க விளைவுகளும் ஏற்படாது.

இந்த ஆராயச்சியில் பதிவு செய்யப்படும் என்னுடைய அனைத்து விவரங்களுயும் மிகவும் இரகசியமாக வைக்கப்படும் என்பதையும், என்னுடைய பெயர் எந்த கட்டுரையிலும் அல்லது வெளியிடுகளிலும் உபயோகப்படுத்தப்படமாட்டாது என்பதையும் அறிவேன்.

இந்த ஆராய்ச்சியின் செய்முறைகளைப் பற்றி கேள்வி கேட்க எனக்கு சந்தர்ப்பம் அளிக்கப்பட்டது. இந்த ஆய்வு தொடர்பாக ஏதாவது சந்தேகம் வந்தால் டாக்டர் நிஹால் தாமஸ் அல்லது இந்த துறையில் வேலை செய்பவர் எவரையும் தொடர்பு கொள்ளலாம் என்பது எனக்கு தெரியும்.

எந்த ஒரு நிபந்தனையும் இல்லாமல் எந்த நேரத்திலும் இந்த ஆராய்ச்சியிலிருந்து நான் விலகிக் கொள்ளலாம் என்பதை அறிவேன். இந்த ஆய்வில் பங்கு பெற எனக்கு பணம் கொடுக்கப்படாது என்பதையும் தெரிந்திருக்கிறேன். மேலும் இதற்காக நான் எந்த பண செலவும் செய்யத் தேவையில்லை என்பதையும் அறிவேன்.

என்னிடத்திலிருந்து எடுக்கப்படும் இரத்த பரிசோதனைகளின் முடிவுகளோ அல்லது அதனை கொண்டு பெறப்பட்ட விவரக்குறிப்புகளோ பின்னாளில், அகச்சுரப்பியல் துறையின் மற்ற ஆய்வுகளுக்காக பயன்படுத்த, சம்மதத்தை அளிக்கின்றேன்.

இந்த ஒப்பந்தத்தின் மூலம் இந்த ஆராய்ச்சியில் கலந்து கொள்ளவும், எனக்கு செய்யப்படும் செய்முறைகளுக்கு என்னை உடப்படுத்திக் கொள்ளவும் நானே விரும்பி ஒப்புதல் அளிக்கின்றேன்.

ஒப்புதல் அளிப்பவர் கையொப்பம்

தேதி

(இந்த ஒப்பந்தத்தில் ஏதாவது புரியவில்லை என்றால், இதில் சம்மந்தப்பட்ட நபரிடம் மேலும் கேட்டு தெரிந்துக்கொள்ளலாம்).

அகச்சுரப்பியல், நிரிழிவு நோய் மற்றும் மெட்டபாலிசம் துறை கிருத்துவ மருத்துவக் கல்லூரி, வேலூர்

பங்கு பெறுவோருக்கான விவரங்கள்

பிறந்த குழந்தையின் விவர விருப்ப படிவம்

ஆய்வின் தலைப்பு: நிரிழிவு நோயால் பாதிக்கப்பட்ட கர்ப்ப பெண்களின் மரபணு மாற்றங்களைக் குறித்த ஆராய்ச்சி

தலைமை ஆராயச்சியாளர்: டாக்டர் நிஹால் தாமஸ்

முதன்மை ஆராயச்சியாளர்

நான், நோயாளி தகவல் படிவத்தை படித்து இந்த ஆராய்ச்சியை குறித்த விவரங்களை நன்றாக அறிந்துக்கொண்டேன்.

1. என்னுடைய குழந்தை இந்த ஆய்வின் தேவைகளுக்கு பொருந்தியிருப்பதால் இந்த ஆய்வில் என்னுடைய குழந்தையை உட்படுத்த விருப்பத்தை தெரிவித்துக்கொள்கிறேன்.
2. என்னில் ஏற்பட்டு இருக்கும் மரபணு மாற்றம் என் குழந்தைக்கும் உள்ளதா என்று கண்டு அறிய என் குழந்தையிடம் இருந்து 1 மிலி இரத்தம் எடுத்துக்கொள்ள அனுமதியளிக்கின்றேன்.
3. இதற்காக எந்தவிதமான பண சலுகை அளிக்கப்படாது என்றறிவேன்.
4. இந்த ஆயிவினால் அறியும் எதிர்மறையான முடிவுகளுக்கு எந்த விதமான பணமும் தரப்படாது என்றறிவேன்.
5. இந்த ஆய்விற்கு சம்மதம் அளிக்காமல் இருப்பதற்கும் விலகிக்கொள்வதற்கும் எனக்கு உரிமையுண்டு என்றறிவேன். மேலும், என் குழந்தைக்கு தரப்படும் சிகிச்சை எந்தவித மாற்றமில்லாமல் தொடர்ந்து பெற்றுக்கொள்ளலாம் என்பதை அறிவேன்.
6. எல்லா தகவல்களும் இரகசியமாக வைக்கப்படும் என்பதறிவேன்.
7. ஆய்வாளரிடம் தொடர்பு விவரங்களை தெரியும்.
8. இந்த விவரங்கள் எனக்கும் புரியும் மொழியில் உள்ளது.
9. நான் இந்த ஆய்விலிருந்து விலகிக் கொண்டாலும் என்னுடைய குழந்தையின் விவரங்களை என்னுடைய அனுமதியில்லாமல் ஆராய்ச்சியாளரும் நிறுவன ஒழுங்கு நெறி குழுவும் உபயோகப்படுத்தலாம் என்பதை அறிவேன்.

10.என் குழந்தையின் மருத்துவ தகவல்களை மற்ற நபர்களுக்கு அளிக்கவோ, வெளியிடவோ தரப்படாது என்பதறிவேன்.

என்னுடைய குழந்தையை இந்த ஆயிவில் உட்படுத்த (பெயர்) ..... ஆகிய நான் சம்மதிக்கிறேன்.

கையொப்பம் / கைரேகை .....

பெயர் குழந்தையோடு உள்ள உறவு .....

தேதி:

ஆராய்ச்சியாளர் கையொப்பம் .....

பெயர் :

தேதி :

சாட்சியாளர் கையொப்பம் .....

பெயர் :

தேதி :

இந்த பிரதியை நீங்களே வைத்துக்கொள்ளலாம்.

மேற்கொண்டு விவரம் அறிய கீழ்கண்ட நபரை அணுகலாம்:

டாக்டர் நிஹால் தாமஸ்

தலைமை பேராசிரியர்

அகச்சுரப்பியல், நிரிழிவு நோய் மற்றும் மெட்டபாலிசம் துறை

கிருத்துவ மருத்துவக் கல்லூரி, வேலூர். தமிழ்நாடு, இந்தியா.

தொலைப்பேசி எண்: 0416-2282694
0416-4200844

அகச்சுரப்பியல், நிரிழிவு நோய் மற்றும் மெட்டபாலிசம் துறை

கிருத்துவ மருத்துவக் கல்லூரி, வேலூர்

பங்கு பெறுவோருக்கான விவரங்கள்

கர்பக்காலத்தில் தோன்றும் நிரிழிவு நோய்க்கான மரபணு ஆராய்ச்சி

தலைமை ஆராய்ச்சியாளர்: டாக்டர் நிஹால் தாமஸ்

நீங்கள் ஒரு ஆய்வில் பங்குப்பெற கேட்டுக் கொள்ளப்படுகிறீர்கள். இந்த ஆய்வில் பங்குபெறுவது தங்களின் விருப்பத்தை சார்ந்தது. இதில் பங்கு பெறாததால் உங்களுக்கு தரப்படும் மருத்துவ தரம் எந்த விதத்திலும் இம்மருத்துவமனையில் குறையாது என்பதை தெரிவித்துக்கொள்கிறேன்.

இந்த ஆய்வை நடத்தும் ஆராய்ச்சியாளர் இந்த ஆய்வின் முக்கியத்துவத்தையும், இதை நடத்தும் விதத்தையும், உங்களின் பங்களிப்பையும் குறித்து விவரமாக கூறுவார். கீழ்கண்ட பாடத்தில் இந்த ஆய்வைக்குறித்து விளக்கப்பட்டுள்ளது. இந்த ஆய்வில் சேருவதற்கு முன்பு உங்களுக்கு ஏதாவது விளங்காவிட்டால் அதை கேட்டுத் தெரிந்துக்கொள்ளவும்

இந்த ஆய்வின் நோக்கம் என்ன?

இந்த ஆய்வானது உங்களுக்கு சர்க்கரை நோய் ஏன் கர்பக்காலத்தில் ஏற்பட்டது என்று கண்டுபிடிக்க நடத்தப்பட்டுள்ளது. சர்க்கரை நோய் இருப்பவர்களுக்கு பிறப்பிலேயே சில பாதிப்புகள் இருக்கிறது. இந்தியாவில் எந்த வகையான பரம்பரை பாதிப்புகள் பொதுவாக காணப்படுகிறது என்பது தெரியவில்லை. கர்பக்காலத்தில் சர்க்கரை நோய் வருவதற்கு எந்த வகையான மரபணு காரணமாக உள்ளது என்பதை கண்டுபிடிக்க இந்த ஆய்வு நடத்தப்படுகிறது.

கீழ்கண்ட மரபணுக்கள் உள்ளதா என்று தீர்மானித்து உள்ளோம்

HNF1A, HNF4A, GCK, HNF1β, IPF1, NEUROD1, KLF11, CEL, PAX4 & INS MODY GENES AND TCF7L2 POLYMORPHISM

இந்த ஆய்வுக்கு தேவை என்ன?

மரபணு பாதிப்பை கண்டுபிடிக்க தேவையான உங்களின் மாதிரி இரத்தம் **(SAMPLE)**

இந்த ஆய்வில் பங்குபெறுவதால் என்ன நன்மை?

நீங்களும் எந்த வகையான மரபணு இந்த சர்க்கரை நோயை ஏற்படுத்தியிருக்கிறது என்பதை தீர்மானிக்க முடியும். இதன் மூலம் மரபணுவை சரிசெய்வதற்கான சிகிச்சையும் ஆலோசனையும் குடும்பத்தினருக்கு அளிக்க முடியும்.

இந்த ஆய்வில் பங்கு பெறுவதால் ஏதாவது பாதிப்பு ஏற்ப்படுமா?

இந்த ஆய்வில் பங்கு பெறுவதால் உங்களுக்கும் உங்கள் குழந்தைக்கும் எந்த விதமான பக்க விளைவுகளும் ஏற்படாது. மேலும் பங்கு பெற்றாலும் இல்லாவிட்டாலும் உங்களுக்கு தரப்படும் சிகிச்சையின் தரம் பாதிக்கப்படாது.

உங்களின் இரத்தம் எவ்வாறு பாதுகாக்கப்படுகிறது?

தங்களின் மாதிரி இரத்தம் கிருத்துவ மருத்துவமனையில் நீரிழிவு நோய் துறையில் -70'சி பாதுகாப்பாக வைக்கப்படும்.

இதில் தரப்படும் விவரங்கள் புரியாவிட்டாலோ அல்லது தேவையான அளவு இல்லாவிட்டாலோ நீங்கள் கேட்டுத்தெரிந்துக்கொள்ளலாம்.

இந்த பிரதியை நீங்களே வைத்துக்கொள்ளலாம்.

மேற்கொண்டு விவரம் அறிய கீழ்கண்ட நபரை அணுகலாம்:

டாக்டர் நிஹால் தாமஸ்

தலைமை பேராசிரியர்

அகச்சுரப்பியல், நீரிழிவு நோய் மற்றும் மெட்டபாலிசம் துறை

கிருத்துவ மருத்துவக் கல்லூரி, வேலூர்.

தமிழ்நாடு, இந்தியா.

தொலைப்பேசி எண்: 0416-2282694
0416-4200844

Proforma for Screening for common MODY Genetic mutations and TCF7L2 in Pregnancy complicated with Diabetes Mellitus

Department of Endocrinology, Diabetes and Metabolism

Christian Medical College, Vellore.

Patient code No:

Proforma date:

Name :

Age :

Hospital no :

Address :

Phone number:

\Obstetric Score:

G		P		L		A		SB	

Period of gestation at first visit:

LMP: EDD: Past

Obstetric history:

	GDM (Y/N)	Outcome (NVD/LSCS)	Baby wt	Gestation (Term/ PT)	Others (if any)
Pregnancy 1					
Pregnancy 2					
Pregnancy 3					

Pregnancy complications- PIH/ polyhydramnios/ oligohydramnios/ IUGR

<u>Current pregnancy</u>

PREGDM/GDM:
Ketosis at diagnosis: Yes/ No/ Not known
Age at onset of diabetes:
Duration of diabetes:
Treatment at diagnosis: OHA/ Insulin/ both/ none
Drug intake: Endocrinopathy:
Yes/ No
Osmotic symptoms : Polyuria/ polydipsia/ weight loss
Gestation age at diagnosis:
Pre-conceptional

weight: Current

weight:

Height:

IBW BMI:

Eclampsia/
preeclamsia

Disease course, glycemic control, complications, And clinical examination co morbidities	Obesity/ hypertension/ dyslipidemia/ PCOS/	
Associated clinical features	H/o pancreatic disease Deafness / blindness Genitourinary anomaly	Y/N Y/N Y/N
Current Clinical		
Current medications and		

Family History (with age of onset of diabetes and treatment)
PEDIGREE CHART TO BE DRAWN HERE

	Type 1 DM	Type 2 DM	Age at onset	Duration	Treatment; Insulin(Y/N) DURATIO	Gestational diabetes	Hypertension	OTHERS

Examination

Weight		Height		BMI	
Acanthosis nigricans	Y/N	Dysmorphism	Y/N	Lipodystrophy	Y/N
Blood pressure		Vision		Hearing	
Systemic Examination Findings	CVS Pulmonary GIT CNS				
Cushingoid features	Y/N	F/o Acromegaly			

Complications of Diabetes

Neuropathy	Y/N
Nephropathy	Y/N
Retinopathy	Y/N
Macrovascular disease	Y/N

Others (if any)–

Investigation reports
Initial blood sugar:
AC/PC
OGTT (0 hr / 1 hr/ 2 hr)-

Others (if any)-

Hb		S. Creatinine	C-peptide (Fasting)		HbA1c	
Proteinuria	Micro/ macro/ nil	GAD titre	POSITIVE./ NEGATIVE			

PREGNANCY OUTCOME :

NVD/
LSCS
Baby
weight :
Gestatio
nal age:
Shoulder dystocia or birth injury, major malformation
Need for intensive neonatal care
(Yes, No) Reason
Neonatal hypo/ hyperglycemia
Hyperbilirubinemia

Postnatal(6 weeks) glycemic status of mother:

ANNEXURE-III: Master-sheet

Sl.No	1	2	3	4	5	6	7	8	9
SAMPLE CODE	MG103	MG40	MG68	MG07	MG22	MG05	MG14	MG33	MG02
CMC HOSPITAL NO:	284998F	103268F	174856F	954303D	968378D	282832F	201018F	922633D	226219F
MODY GENE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	#KLF
MUTATION	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	Q62R, Gln62Arg
AGE	27	24	25	30	20	30	35	25	28
AGE AT DIAGNOSIS	27	24	25	30	20	30	35	25	28
DURATION OF DIABETES	NA	NA	NA	NA	NA	NA	NA	NA	NA
GESTATIONAL AGE AT DIAGNOSIS	12 weeks	16 weeks	32 weeks	24 weeks	28 weeks	20 weeks	12 weeks	8 weeks	28 weeks
TYPE OF DIABETES	GDM	GDM	GDM	GDM	GDM	GDM	GDM	GDM	GDM
NO. OF GENERATIONS WITH DM	3	2	2	2	3	2	1	3	2
FAMILY MEMBERS WITH DIABETES	pts father & moth; pts fat's sis-40 and father; pts mot sis-35 age	patients parents	patients parents and pts mother side sisters	Parents	father and fat mother	pts father; pts fat's mother and sis-2 and brother-	none	pts sis-2 years back, pts fath-55 and fat's mother and pts mother's fat-55 & moth65	fat sis and mother bro
G	1	1	2	1	1	3	1	1	1
P	0	0	0	0	0	1	0	0	0
L	0	0	0	0	0	1	0	0	0
MISCARRIAGE/MTP/IUD	0	0	1	0	0	1	0	0	0
FSB/DELIVERED	2	1	1	1	1	UK	1	1	1
DELIVERY place	CMC	CMC	CMC	EI	CMC	EI	CMC	CMC	CMC
INSULIN	Y	Y	Y	N	N	Y	N	N	N
Weight (kgs)	78	59	65	55	64	58	60	54	46
Height (cms)	157	149	157	153	150	151.5	160	150	152
BMI (kg/m^)	31.64428577	26.57537949	26.37023814	23.49523687	28.44444444	25.26985372	23.4375	24	19.9099723

ANNEXURE-III: Master-sheet

b_wt	2.01 2.01	3.12	3.01	2.7	2.78	NA	3.1	2.84	3.18
sex	2	1	1	1	1	NA	2	1	1
apgar	NA	9,9	NA	NA	9&10	NA	9&9	9,9	9,9
weeks	36+1	39+2	38+5	37	36	NA	38	38+6	39
term	NO	Y	Y	Y	NO	NA	Y	Y	Y
preterm	Y	NO	NO	NO	Y	NA	NO	NO	NO
nvd	Y	Y	Y	y	Y	NA	NO	NO	Y
lscs	NO	NO	NO	NO	NO	NA	Y	Y	NO
IUGR	NO	NO	NO	NO	NO	NA	NO	NO	NO
IUD	NO	NO	NO	NO	NO	NA	NO	NO	NO
AC	120	106	NA	107	90	NA	80	81	76
PC	156	157	NA	133	112	NA	163	191	116
OGTT-0	130	NA	114	93	82	101	NA	91	NA
OGTT-1HR	NA	NA	216	NA	181	196	NA	NA	NA
OGTT-2 HRS	NA	NA	187	108	170	182	NA	229	NA
HBA1C	6.1	NA	NA	4.4	NA	NA	5.8	6.4	5.2
Date of delivery	02/03/2013	30.07.2012	30.08.12	09/12/2011	03/08/2012	Mar-13	28/11/2012	28/11/11	17/08/2012
ORAL AGENTS	Metformin	Daonil	metformin	daonil	daonil	metformin	metformin	metformin	NO
AC_POSTDELI	NA	NA	NA	NA	NA	NA	NA	NA	NA
PC_PD	NA	NA	NA	NA	NA	NA	NA	NA	NA
A1C_PD	NA	NA	NA	5.3	NA	NA	NA	NA	NA
Therapy	NA	NA	NA	DIET	NA	NA	NA	NA	NA
COMMENTS:prev delivery/complications	she had Atonic PPH(blood loss-1.5L) and received 3 pints of blood post natally.	FORCEPS	Suction Cup	NONE	NONE	G1LSCS; GDM-3.45kg, term; G2 abortion at 40 days	NONE	NONE	NONE

ANNEXURE-III: Master-sheet

Sl.No	10	11	12	13	14	15	16	17	18
SAMPLE CODE	MG29	MG93	MG15	MG46	MG102	MG87	MG10	MG24	MG30
CMC HOSPITAL NO:	138568B	424242D	736914D	263786F	452984D	386412F	016299F	802680B	300849F
MODY GENE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	CEL	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE
MUTATION	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	F83S Phe83Ser	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
AGE	36	26	25	26	27	24	29	37	34
AGE AT DIAGNOSIS	36	26	25	26	27	24	29	37	34
DURATION OF DIABETES	NA	NA	NA	NA	NA	NA	NA	NA	NA
GESTATIONAL AGE AT DIAGNOSIS	20 weeks	16 weeks	8 weeks	20 weeks	8 weeks	12 weeks	28 weeks	24 weeks	11 weeks
TYPE OF DIABETES	GDM	GDM	GDM	GDM	GDM	GDM	GDM	GDM	GDM
NO. OF GENERATIONS WITH DM	2	3	3	1	3	3	2	2	2
FAMILY MEMBERS WITH DIABETES	fath, moth's sis and brots	pts father and mother; pts mother's brother and mother's parents, pts father's sis-2 and bro-1	fath, fath bro, moth mothers	none	sister, father, mother, father's sisters and mother's brothers	mother, moth father	mother	pts father and pt mother's brother	pts brother-33 and mother-60
G	5	1	2	1	4	1	1	6	3
P	2	0	1	0	1	0	0	1	1
L	2	0	1	0	1	0	0	1	1
MISCARRIAGE/MTP/IUD	2	0	0	0	MTP2	0	0	4	1
FSB/DELIVERED	1	1	1	1	1	1	1	1	1
DELIVERY place	CMC	CMC	CMC	CMC	EI	CMC	CMC	CMC	EI
INSULIN	Y	N	N	N	Y	N	N	N	N
Weight (kgs)	75	75	64	54	76	62	52	55	61
Height (cms)	150	152	168	146	158	156	150	152	156
BMI (kg/m^)	33.33333333	32.46191136	22.67573696	25.33308313	30.44383913	25.47666009	23.11111111	23.80540166	25.06574622

ANNEXURE-III: Master-sheet

b_wt	3.15	3.04	3.2	3.08	2.8	3.48	2.22	2.54	NA
sex	1	2	1	1	2	1	1	2	NA
apgar	9&10	9,9	9&10	9&9		9&10	8&9	9&9	NA
weeks	38	38+6	40	37+6	37	39	35+4	38+5	24+1
term	Y	Y	Y	y	Y	Y	NO	Y	NO
preterm	NO	NO	NO	NO	NO	NO	Y	NO	Y
nvd	Y	Y	Y	NO	NO	NO	Y	NO	Y
lscs	NO	NO	NO	Y	Y	Y	NO	Y	NO
IUGR	NO	NO	NO	NO	NO	NO	NO	NO	NO
IUD	NO	NO	NO	NO	NO	NO	NO	NO	Y
AC	112	107	91	95	NA	NA	103	107	101
PC	127	143	128	179	133	NA	175	160	142
OGTT-0	NA	NA	103	NA	99	NA	NA	92	NA
OGTT-1HR	NA	NA	NA	NA	NA	NA	NA	181	NA
OGTT-2 HRS	NA	NA	131	NA	147	NA	NA	157	NA
HBA1C	6	6	5.8	5.2	5.6	5.6	NA	5.5	4.8
Date of delivery	03/07/2012	12/08/2012	02/11/2012	02/02/2013	04/09/2013	27/03/2013	24/12/2011	01/08/2012	14/12/2012
ORAL AGENTS	metformin	METFORMIN	diet	metformin	METFORMIN	metformin	METFORMIN	METFORMIN	METFORMIN
AC_POSTDELI	NA	90	103	NA	NA	NA	NA	NA	NA
PC_PD	NA	116	139	NA	NA	NA	NA	NA	NA
A1C_PD	NA	NA	NA	NA	NA	NA	NA	NA	NA
Therapy	NA	NA	NA	NA	NA	NA	NA	NA	NA
COMMENTS:prev delivery/complications	previous GDM ; G1 3.5 kg; G2 2.8 kg	NONE	G1 - NVD 3.25 kgs, GDM term	NONE	G1-LSCS -2.9KG-TERM-MALE.	NONE	NONE	G1:Spontaneous abortion at 3 months. G2: 2005/ 40+6/ CMC/ LSCS/ arrest of descent/boy/2.9kg/ A&W. G3: Spontaneous abortion at 3 months, D/C done. G4: Spontaneous abortion, D/C done. G5: Spontaneous abortion, D/C done.G6: Present pregnancy.	G1: 9 years back underwent LSCS for failed induction delivered a girl baby 2.42 kg. G2: spontaneous abortion at 4 months. G3 :ABORTED AT 24 WEEKS (6 MONTHS)

ANNEXURE-III: Master-sheet

Sl.No	19	20	21	22	23	24	25	26	27
SAMPLE CODE	MG104	MG65	MG35	MG44	MG88	MG48	MG86	MG39	MG27
CMC HOSPITAL NO:	033350D	201167D	153089f	050578F	148280F	780375d	716323B	061531F	202100F
MODY GENE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NEUROD1	NOT APPLICABLE	NOT APPLICABLE
MUTATION	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	E59Q Glu59Gln	NEGATIVE	NEGATIVE
AGE	26	31	29	20	23	24	37	31	25
AGE AT DIAGNOSIS	26	31	29	20	23	24	37	31	25
DURATION OF DIABETES	NA	NA	NA	NA	NA	NA	NA	NA	NA
GESTATIONAL AGE AT DIAGNOSIS	32 weeks	12 weeks	12 weeks	20 weeks	12 weeks	12 weeks	24 weeks	20 weeks	20 weeks
TYPE OF DIABETES	OVERT	OVERT	OVERT	OVERT	OVERT	OVERT	OVERT	OVERT	OVERT
NO. OF GENERATIONS WITH DM	2	2	1	2	3	2	3	3	3
FAMILY MEMBERS WITH DIABETES	pts mother and moth's sis-3 and bro-3	pts - bro-2 & sis-1 and father; pts fat's bro-1 sis-3 & mot	pts sister	patient's parents	patient's father and his sisters-2 & bro-1; patient's mother and mother's sisters-2and brother-1; patient's motherside	pts sis-1 and father	pts sis-37 and parents;	pts father and mother; pts fat's sister; pts mother's sis-2 and bro-1 and mother's mother	pts mother's and mother's sis-2 and bro-2 and mother's mother
G	3	3	2	1	1	2	1	1	1
P	2	1	1	0	0	1	0	0	0
L	1	Y	1	0	0	1	0	0	0
MISCARRIAGE/MTP/IUD	IUD1	1	0	0	0	0	0	0	0
FSB/DELIVERED	1	1	FSB1	1	1	UK	1	1	1
DELIVERY place	CMC	EI	CMC	CMC	CMC	EI	CMC	CMC	CMC
INSULIN	Y	Y	Y	Y	Y	Y	Y	Y	Y
Weight (kgs)	58	61	64	62	65	64	83	93	58
Height (cms)	155	149	160	155	148	155	160	170	145
BMI (kg/m^)	24.14151925	27.47623981	25	25.80645161	29.67494522	26.63891779	32.421875	32.1799308	27.5862069

ANNEXURE-III: Master-sheet

b_wt	2.94	NA	1.88	2.72	2.78	NA	3.7	2.9	2.92
sex	1	NA	1	1	1	NA	1	2	1
apgar	9,10	NA	9,9	9 &10	9,9	NA	9&9		9&10
weeks	39+2	NA	37	38+2	38+3	NA	37+4	38+2	38
term	Y	NA	Y	Y	Y	NA	Y	Y	Y
preterm	NO	NA	NO	NO	NO	NA	NO	NO	NO
nvd	Y	NA	Y	Y	NO	NA	NO	NO	NO
lscs	NO	NA	NO	NO	Y	NA	Y	Y	Y
IUGR	NO	NA	Y	NO	NO	NA	NO	NO	NO
IUD	Y	NA	NO	NO	NO	NA	NO	NO	NO
AC	109	NA	145	205	141	146	151	NA	159
PC	148	NA	200	321	197	255	189	NA	164
OGTT-0	NA	163	NA	NA	NA	279	119	128	NA
OGTT-1HR	NA	305	NA	NA	NA	NA	229	277	NA
OGTT-2 HRS	NA	315	NA	NA	NA	NA	172	213	NA
HBA1C	6.8	8.4	7.4	9.6	6.9	7.2	NA	7.1	NA
Date of delivery	17/05/2012	2012	21.09.2012	18/04/2012	13/09/2012	Apr-12	13/03/2012	11/03/2012	14/12/2012
ORAL AGENTS	NO	Metformin	Daonil & Metformin	Glyciphage	Metformin	NO	NO	NO	metformin
AC_POSTDELI	89	NA	121	236	NA	NA	197	120	131
PC_PD	115	NA	185	285	NA	NA	262	123	157
A1C_PD	NA	NA	5.6	12.5	NA	NA		NA	NA
Therapy	NA	Metformin	Metformin	oral	NA	NA	oral	NA	diet
COMMENTS:prev delivery/complications	HYPOTHYROIDISM	G1 - FT LSCS (CPD), Birth weight 3.186 kg. G2- miscarraige @ 6 months.	G1: prinary infertility concieved spontaneously. 2 years back, delivered a FSB female child, 1.25 kg normally.	NONE	NONE	G1 - 15/09/10, at 8 months scrub typhes with ARDs. she was admitted in MHDU. Preterm vaginal delivery girl, 1.75 kg.	NONE	NONE	NONE

ANNEXURE-III: Master-sheet

Sl.No	28	29	30	31	32	33	34	35	36
SAMPLE CODE	MG19	MG92	MG42	MG94	MG43	MG96	MG95	MG26	MG23
CMC HOSPITAL NO:	321162C	258224F	906625C	084291F	144881F	428266F	110953F	250238B	221895F
MODY GENE	PDX	NOT APPLICABLE	INS	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	GCK	NOT APPLICABLE	NOT APPLICABLE
MUTATION	H94Q His94Gln	NEGATIVE	G44R Gly44Arg	NEGATIVE	NEGATIVE	NEGATIVE	I348F Ile348Phe	NEGATIVE	NEGATIVE
AGE	35	21	35	25	30	28	23	36	32
AGE AT DIAGNOSIS	35	21	35	25	30	28	23	36	32
DURATION OF DIABETES	NA	NA	NA	NA	NA	NA	NA	2 MONTHS	12 MONTHS
GESTATIONAL AGE AT DIAGNOSIS	5 weeks	12 weeks	28 weeks	8 weeks	20 weeks	12 weeks	21 weeks	NA	NA
TYPE OF DIABETES	OVERT	OVERT	OVERT	OVERT	OVERT	OVERT	OVERT Previous GDM	PREGDM	PREGDM
NO. OF GENERATIONS WITH DM	3	2	3	3	1	3	3	3	3
FAMILY MEMBERS WITH DIABETES	pts parents; pts moth's brother; pts husb's mother	pts mother's mother	pts father-65 and fat's bro-72	pts parents, pt mother's brother and mother; pt father's father and sis-1 and bro-2	none	father, mother, fath's mother and brothers and mother's mother, moth sis and bro	pts parents and mot's sister and fath's broth and father	mother, moth father, moth sis's and brother	pt's motherside uncle, mother's father; pt fathers bro-2
G	4	1	2	2	3	1	2	4	2
P	2	0	1	0	0	0	1	2	1
L	1	0	1	0	0	0	1	2	1
MISCARRIAGE/MTP/IUD	1, END1	0	0	1	2	0	0	1	0
FSB/DELIVERED	1	1	1	1	1	1	1	1	1
DELIVERY place	CMC	CMC	CMC	CMC	CMC	CMC	CMC	CMC	CMC
INSULIN	Y	Y	Y	Y	Y	Y	Y	Y	Y
Weight (kgs)	79	70	76	64	69	81	54	95	87
Height (cms)	164	154	157	155	154	156	154	167	157
BMI (kg/m^)	29.37239738	29.51593861	30.83289383	26.63891779	29.09428234	33.28402367	22.76943835	34.06360931	35.29554952

ANNEXURE-III: Master-sheet

b_wt	3.3	2.99	2.87	3.5	2.22	1.99	3.14	2.47	2.8
sex	1	2	2	1	2	1	2	2	1
apgar	9,10	9&9	9,10	9,10	9&9	9&10	9,10	9,10	9&9
weeks	36+4	37+1	38+2	37+3	37+6	31	38+2	37+6	38+3
term	NO	Y	Y	Y	Y	NO	Y	Y	Y
preterm	Y	NO	NO	NO	NO	Y	NO	NO	NO
nvd	NO	Y	Y	NO	Y	Y	Y	Y	NO
lscs	Y	NO	NO	Y	NO	NO	NO	NO	Y
IUGR	NO	NO	NO	NO	NO	NO	NO	NO	NO
IUD	NO	NO	NO	NO	NO	NO	NO	NO	NO
AC	172	147	126	NA	158	NA	NA	126	217
PC	226	235	156	NA	256	NA	NA	131	368
OGTT-0	NA	NA	183	115	NA	NA	123	NA	NA
OGTT-1HR	NA	NA	382	270	NA	NA	229	NA	NA
OGTT-2 HRS	NA	NA	359	267	NA	NA	201	NA	NA
HBA1C	6.4	9.3	7.3	5.5	NA	7.8	7	6.8	
Date of delivery	23/1/2012	23/01/2013	28/12/2012	25/2/2012	01/06/2012	NA	23/11/2013	28/07/2013	10/09/2012
ORAL AGENTS	NO	NO	METFORMIN	metformin	metformin	NO	diet	metformin	metformin
AC_POSTDELI	NA	126	NA	NA	NA	NA	NA	91	146
PC_PD	NA	264	NA	NA	NA	NA	NA	142	179
A1C_PD	NA	NA	NA	6.5	NA	NA	NA	NA	NA
Therapy	NA	NA	NA	insulin	NA	NA	NA	NA	metformin
COMMENTS:prev delivery/complications	G1-1 kg lscs-32 weeks,previous GDM G2-lscs-36 weeks 1.7 kgs; G3: Present pregnancy, hypertension;	NONE	G1- Term, Baby boy, now alive and well.	BOOKED G2A1 AT 37+3 WEEKS DELIVERED VAGINALLY BY LOW FORCEPS A SINGLE LIVE BABY BOY 3.59 KG APGAR 9/10 ON 25/2/2012 AT 10:43 AM.	G1:2008,spontaneous abortion at 3 months,D&C done; G2:2011,spontaneous abortion at 2 months,D&C done	BILATERAL PDR [LASER]	G1: 2012- GDM on insulin. FTNVD in CMC. Male baby, 3.14Kgs,	G1-abortion; G2-3.8kg-girl-term-nvd; G3-male-3.8-term-gdm hypertension-2 years	G1 - 1998 RUHSA -emergency LSCS for post dates and delivered a male child with a birth weighth of 3.45kg,

ANNEXURE-III: Master-sheet

Sl.No	37	38	39	40	41	42	43	44	45
SAMPLE CODE	MG31	MG66	MG41	MG63	MG62	MG28	MG101	MG53	MG56
CMC HOSPITAL NO:	399291F	916641D	206916f	597217D	453358D	958747D	181517B	619107D	081270F
MODY GENE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NEUROD1	PDX	HNF1A KLF	NOT APPLICABLE
MUTATION	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	F318S Phe318Ser	E224K , Glu224Lys	S3C Ser3Cys Q62R	NEGATIVE
AGE	23	28	36	34	31	29	26	25	29
AGE AT DIAGNOSIS	23	28	33	31	28	29	10	22	28
DURATION OF DIABETES	2 MONTHS	24 MONTHS	36 MONTHS	36 MONTHS	36 MONTHS	1 MONTH	16 YEARS	36 MONTHS	12 MONTHS
GESTATIONAL AGE AT DIAGNOSIS	NA	NA	NA	NA	NA	NA	NA	NA	NA
TYPE OF DIABETES	PREGDM	PREGDM	PREGDM	PREGDM	PREGDM	PREGDM	PREGDM	PREGDM	PREGDM
NO. OF GENERATIONS WITH DM	3	3	2	2	3	3	1	3	3
FAMILY MEMBERS WITH DIABETES	father and mother, fath fat and moth fat	father, mother mother mot and sis	pts sister-33 and father's brother	pts father	pts's hus's sis and father; pts father's mother and bro	pts brother, pts father and mother, pts father's parents and sis; pts mother's parents	none	mother, father, paternal uncle, aunt grandfather,	pts father; pts father's mother; pts mother's mother
G	1	1	2	3	2	2	1	2	1
P	0	0	1	1	1	1	0	1	0
L	0	0	1	1	1	1	0	0	0
MISCARRIAGE/MTP/IUD	0	0	0	1	0	0	0	0 LND1	0
FSB/DELIVERED	0	1	1	0	1	1	1	1	1
DELIVERY place	CMC	CMC	CMC	CMC	CMC	CMC	CMC	CMC	CMC
INSULIN	Y	Y	Y	Y	Y	Y	Y	Y	Y
Weight (kgs)	51	52	90	85	68	70	53	76	53
Height (cms)	151	151	174	165	154	160	153	160	153
BMI (kg/m^)	22.36744002	22.80601728	29.72651605	31.22130395	28.67262608	27.34375	22.64086462	29.6875	22.64086462

ANNEXURE-III: Master-sheet

b_wt	NA	2.5	2.94	1.62	2.064	3.1	2.24	3.94	2.46
sex	NA	2	2	1	2	2	1	2	1
apgar	NA	7&9	9&9	NA	8&9	9,9	9&10	9,10	9,10
weeks	8+5	38+2	36+4	34+6	37+3	38	36+3	37+5	39+1
term	NO	Y	Y	NO	Y	Y	NO	Y	Y
preterm	Y	NO	Y	Y	NO	NO	Y	NO	NO
nvd	Y	NO	NO	Y	NO	NO	Y	NO	NO
lscs	NO	Y	Y	NO	Y	Y	NO	Y	Y
IUGR	NO	NO	NO	y	NO	NO	NO	NO	NO
IUD	NO	NO	NO	NO	NO	NO	NO	NO	NO
AC	NA	NA	113	NA	96	97	NA	154	98
PC	240	NA	184	NA	147	162	NA	251	200
OGTT-0	NA	NA	NA	NA	NA	NA	NA	163	NA
OGTT-1HR	NA	NA	NA	NA	NA	NA	NA	272	NA
OGTT-2 HRS	NA	NA	NA	NA	NA	NA	NA	288	NA
HBA1C	10.6	8.9	7.7	10.2	9.5	8.7	8.8	6.4	8.5
Date of delivery	11/02/2013	11/10/2012	15.11.2012	27/03/2012	30/08/2012	23/01/2013	13/01/2012	23/12/2011	15/05/2012
ORAL AGENTS	METFORMIN	metformin	Metformin	metformin	metformin	METFORMIN	NO	NO	NO
AC_POSTDELI	NA	NA	NA	NA	NA	132	NA	174	NA
PC_PD	NA	NA	NA	NA	NA	200	NA	195	NA
A1C_PD	NA	NA	NA	10.8	NA	6.8	7.1	9.5	NA
Therapy	NA	NA	NA	insulin	NA	GLYNASE & METFORMIN	INSULIN	INSULIN	metformin & glimepiride
COMMENTS:prev delivery/complications	BOOKED PRIMIGRAVIDA AT 8+5 WEEKS MISSED ABORTION MVA DONE	EMERGENCY LSCS FOR FAILED INDUCTION	G1:12years, term, NVD, girl,2.7kg,A&W ; hypertension	G1 8years old girl. G2-2009 DM. Aborted at 2 months. G3- again in July 2011 and now is at 16 weeks gestation. macerated baby	G1, LSCS 4.2 KG TERM, GDM, PERSISTENT HIGH SUGARS	G1: PPROM, GDM, 36 weeks , LSCS, boy, 2.02kg , alive and well, hypothyroid	NONE	baby born had anomalies- Sius inversus, RGV, Pulmonary atresia. Died after one month of extrauterine life.	UNDERWENT EMERGENCY LSCS FOR FAILED INDUCTION AND DELIVERED A SINGLE LIVE TERM BABY BOY OF 2.46 KG WITH APGAR 9/10 ON 15/5/12 AT 6.28 PM

ANNEXURE-III: Master-sheet

Sl.No	46	47	48	49	50
SAMPLE CODE	MG73	MG85	MG71	MG32	MG34
CMC HOSPITAL NO:	123639F	386933F	470810C	010292F	146456F
MODY GENE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE	NOT APPLICABLE
MUTATION	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
AGE	27	37	39	30	37
AGE AT DIAGNOSIS	25	34	37	25	36
DURATION OF DIABETES	24	36	24	60	6 MONTH
GESTATIONAL AGE AT DIAGNOSIS	NA	NA	NA	NA	NA
TYPE OF DIABETES	PREGDM	PREGDM	PREGDM	PREGDM	PREGDM
NO. OF GENERATIONS WITH DM	3	3	3	2	2
FAMILY MEMBERS WITH DIABETES	pts father, pts father's parents and sis and bro	moth, fath fath and bro	pts mother; pts moth's father, sis-1 and bro-1; pts husb's mother	moth, moth sists&bro	pts' bro-2; mother
G	2	2	1	2	1
P	1	1	0	1	0
L	1	1	0	1	0
MISCARRIAGE/MTP/IUD	0	0	0	0	0
FSB/DELIVERED	1	1	1	1	1
DELIVERY place	CMC	CMC	CMC	CMC	CMC
INSULIN	Y	Y	Y	Y	Y
Weight (kgs)	71	64	63	72	52
Height (cms)	159	150	146	160	150
BMI (kg/m^)	28.08433211	28.44444444	29.55526365	28.125	23.11111111

ANNEXURE-III: Master-sheet

b_wt	2.77	3	3.1	3.4	1.73
sex	2	2	1	2	1
apgar	9&9	9&9	6&9	9&10	9&9
weeks	37+2	38+1	37+5	36	36+5
term	Y	Y	Y	NO	NO
preterm	NO	NO	NO	Y	Y
nvd	Y	NO	NO	NO	Y
lscs	NO	Y	Y	Y	NO
IUGR	NO	NO	NO	NO	NO
IUD	NO	NO	NO	NO	NO
AC	119	96	131	130	187
PC	130	210	145	207	305
OGTT-0	NA	NA	142	NA	NA
OGTT-1HR	NA	NA	NA	NA	NA
OGTT-2 HRS	NA	NA	225	NA	NA
HBA1C	7.5	7	6.5	8.3	8.1
Date of delivery	05/06/2012	12/08/ 013	22/05/2012	19/2/1913	29/09/2012
ORAL AGENTS	metformin	metformin	metformin	metformin	metformin
AC_POSTDELI	NA	81	134	NA	NA
PC_PD	NA	127	141	NA	NA
A1C_PD	NA	NA	6.3	NA	NA
Therapy	NA	NA	metformin	NA	NA
COMMENTS:prev delivery/complications	G1-3.3kg-term-nvd;	G1:2009 LSCS at term for breech presentation Boy baby 2.6kg A/W	breech	G1:Term, LSCS for failed induction,Pregestational diabetis mellitus on insulin, 3.5Kg baby, alive and well. G2:Present pregnancy.	NONE

MASTER SHEET-II

TCF7L2 GENOTYPE

TCF7L2 GENOTYPE CASES			
CMCH	**rs7903146**	**rs12255372**	**rs4506565**
201167D	TT	TT	TT
103268F	CT	GT	TT
153089f	TT	GT	TT
050578f	CC	GG	AA
148280f	CC	GG	AA
174856f	CC	GG	AA
284998f	TT	GT	TT
317260c	CT	GT	AT
061664d	CT	GT	AT
174346f	CC	GG	AA
780375d	CC	GG	AA
217827f	CT	GT	AT
221895F	CT	GT	AT
293221F	CC	GG	AA
292148F	CC	GG	AA
832173d	CT	GT	AT
080801f	CT	GG	AT
995031B	CC	GG	AT
789623d	CC	GG	AA
001588f	CT	GT	AT
016299f	CT	GT	AT
090917d	CC	GG	AA
095108b	CT	GT	AT
201018F	CC	GG	AA
255521f	CC	GG	AA
110953f	CT	GG	AT
206916f	CT	GG	AT
412079b	CC	GG	AA
426086d	CC	GG	AA

TCF7L2 GENOTYPE CONTROL			
CMC ID	rs7903146	rs12255372	rs4506565
426307D	CC	GG	AA
560837C	CC	GG	AA
426313D	CC	GG	AA
426314D	CC	GG	AA
426303D	CT	GT	AT
432558D	CC	GG	AA
432556D	CC	GG	AA
432560D	CT	GG	AT
432554D	CT	GT	AT
432551D	CC	GG	AA
427832D	CT	GT	Undetermined
100233B	CC	GG	AA
874954B	CC	GG	AA
423871D	CC	GG	AA
423873D	CC	GG	AA
423860D	CT	GT	AT
983882C	CC	GG	AA
434576D	CC	GG	AA
435135D	CT	GG	AT
435139D	CC	GG	AA
422813D	CT	GG	AT
422815D	CC	GG	AA
422816D	CT	GT	AT
422817D	CC	GG	AA
439649D	CC	GG	AA
439653D	CC	GG	AA
435913D	CT	GT	AT
439666D	CC	GG	AA
439661D	CC	GG	AA

MASTER SHEET-II

TCF7L2 GENOTYPE

TCF7L2 GENOTYPE CASES			
CMCH	**rs7903146**	**rs12255372**	**rs4506565**
716323b	CC	GT	AA
922633d	CC	GG	AA
597217d	TT	GT	TT
061531F	CT	GG	AT
734933d	CC	GG	AA
138568b	CT	GG	AT
453358d	CT	GG	AT
202100f	CT	GT	AT
874785d	CT	GT	AT
424242d	TT	TT	TT
958747d	CC	GG	AA
321162c	CT	GG	AT
720400d	CT	Undetermined	AT
102911f	CC	GG	AA
263786f	CT	GG	AT
906625c	TT	GT	TT
619107d	CC	GG	AA
146456f	TT	GT	TT
083982f	CT	GT	AT
084291f	TT	GG	TT
181517b	CC	GG	AA
470810c	CC	GG	AA
802680b	CT	GT	AT
028109f	CT	GT	AT
081270f	CC	GG	AA
236272f	CC	GG	AA
300849f	CC	GG	AA
258224f	CT	GG	AT
235254f	CC	GG	AA
107984c	TT	TT	TT

TCF7L2 GENOTYPE CONTROL			
CMC ID	rs7903146	rs12255372	rs4506565
439660D	CT	GT	AT
435911D	TT	TT	TT
435897D	TT	GT	TT
435903D	CC	GG	AA
435910D	CT	GT	AT
443711D	TT	TT	TT
443722D	CT	GT	AT
443707D	CC	GG	AA
445139D	CT	GG	AT
435902D	CT	GG	AT
448295D	CC	GG	AA
445141D	CC	GG	AA
449385D	CT	GG	AT
449379D	CT	GT	AT
456160D	CC	GG	AA
449379D	CT	GT	AT
449334D	CT	GT	AT
453311D	CC	GG	AA
453323D	TT	GT	Undetermined

MASTER SHEET-II

TCF7L2 GENOTYPE

TCF7L2 GENOTYPE CASES			
CMCH	**rs7903146**	**rs12255372**	**rs4506565**
694042D	CC	GG	AA
122400f	CC	GG	AA
821969d	CC	GG	AA
217995f	CC	GG	AA
229042f	CC	GG	AA
091707f	CC	GG	AA
175714d	CC	GG	AA
099986f	CC	GG	AA
177998f	CT	GT	AT
100972c	CT	GT	AT
080522f	CC	GG	AA
519176d	CC	GG	AA
238713a	CC	GT	AT
634643d	CT	GT	AT
013951F	CC	GG	AA
656966d	CC	GG	AA
362957f	CT	GG	AT
255992f	TT	GT	TT
471988b	TT	TT	TT
736914D	CT	GT	AT
215868f	CT	GT	AT
579433c	CC	GG	AA
716132d	CC	GG	AA
769588d	TT	TT	TT
244460f	CC	GG	AA
230273d	CT	GG	AT
136926f	CT	GT	AT
777756d	CT	GT	AT
228886c	CC	GG	AA
243150f	CC	GG	AA

MASTER SHEET-II

TCF7L2 GENOTYPE

TCF7L2 GENOTYPE CASES			
CMCH	**rs7903146**	**rs12255372**	**rs4506565**
916641D	CT	GT	AT
475208d	CT	GT	AT
234112f	CC	GG	AA
359494d	CT	GG	AT
516241D	CC	GG	AA
680601c	CT	GT	AT
636415d	CT	GT	AT
282832f	CT	GT	AT
070127f	CC	GG	AA
258224f	CT	GG	AT
144881f	CT	GT	AT
100834c	CT	GG	AT
779863d	CC	GG	AA
743843d	CC	GG	AA
994826d	TT	GT	TT
968378d	CC	GG	AA
010292f	CC	GG	AA
691180d	TT	TT	TT
386933f	CT	GG	AT
366207f	CC	GG	AA
425279F	TT	TT	TT
425202f	CT	GT	AT
428144d	CC	GG	AA
411278f	CC	GG	AA
250238B	CT	GT	AT
428266F	CC	GG	AA
361822f	CT	GG	AT
321174c	TT	TT	TT